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EFFECTS OF CETYLTRIMETHYLAMMONIUM BROMIDE ON CATALYTIC PROPERTIES OF KIDNEY MICROSOMAL GLUCOSE-6-PHOSPHATASE, INORGANIC PYROPHOSPHATE–GLUCOSE PHOSPHOTRANSFERASE AND INORGANIC PYROPHOSPHATASE ACTIVITIES

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SUMMARY

The modifying effects of the cationic detergent cetyltrimethylammonium bromide (CTAB) on catalytic properties of kidney microsomal D-glucose-6-*P* phosphohydrolase (EC 3.1.3.9) and associated inorganic pyrophosphatase and PP_i–glucose phosphotransferase activities have been studied. Initial reaction velocities, apparent Michaelis constant values and apparent maximal reaction velocity values were determined at various H^+ concentrations with microsomal preparations which had been supplemented with the detergent to a final concentration (w/v), of 0.00, 0.05, 0.10, 0.20 or 0.30% 15 min prior to assay. Measurements of initial reaction velocities at various assay pH values indicated that CTAB treatment produced a shift in pH optimum of glucose-6-*P* phosphohydrolase activity towards acid pH values and in that of inorganic pyrophosphatase and phosphotransferase activities in the direction of neutrality. Both on the basis of initial reaction velocity values and apparent maximal reaction velocity values extrapolated to infinite substrate concentrations, lower concentrations of CTAB (0.05 and 0.10%, w/v) were found to elevate all activities, while higher concentrations (0.20 and 0.30%, w/v) were less effective in this respect or actually inhibited. In contrast with these polyphasic effects of the detergent on velocity values, apparent Michaelis constant values for all phosphate substrates, assayed at pH 4.5, 5.5, and 6.5, all decreased progressively as CTAB concentrations were increased from 0.05 to 0.30% (w/v). Plots (see J. S. FRIEDENWALD AND G. D. MEANGWYN-DAVIES, in W. D. MCELROY AND B. GLASS, *The Mechanism of Enzyme Action*, Johns Hopkins Press, Baltimore, 1954, p. 180), of reciprocals of apparent Michaelis constant values for these compounds *versus* CTAB concentrations were linear, supporting the concept that CTAB acts as a "coupling" activator at lower concentrations and as a noncompetitive inhibitor at higher concentrations. Apparent Michaelis constants for PP_i—0.3–0.4 mM with 0.30% (w/v) CTAB (pH 5.5)—are the

Abbreviation: CTAB, cetyltrimethylammonium bromide.

lowest such values yet reported. The apparent Michaelis constant value for glucose in the phosphotransferase reaction, in contrast, was only modestly changed by CTAB treatment of microsomes. Several alternative mechanistic interpretations are considered briefly.

INTRODUCTION

In earlier studies in this laboratory[1-4], the cationic detergent cetyltrimethylammonium bromide (CTAB) was found to exert interesting and rather dramatic modifying effects on the action of a variety of inhibitors on both hydrolytic and synthetic (see ref. 3) activities of microsomal D-glucose-6-*P* phosphohydrolase (EC 3.1.3.9). For example, preliminary treatment of microsomes with this detergent markedly potentiated the inhibition of glucose-6-*P* phosphohydrolase activity of the enzyme (Reaction 1) by various nucleotides[1], orthophosphate[1-3] and phlorizin[4,5]. In contrast with these effects with the phosphohydrolase activity, CTAB treatment of microsomal preparations little altered the inhibition by P_i of PP_i–glucose phosphotransferase activity (Reaction 2) of the enzyme[2,3] and significantly ameliorated inhibition by phlorizin of this latter activity[4,5].

$$\text{Glucose-6-}P + H_2O \rightarrow \text{glucose} + P_i \quad (1)$$

$$PP_i + \text{glucose} \rightarrow \text{glucose-6-}P + P_i \quad (2)$$

$$PP_i + H_2O \rightarrow 2\ P_i \quad (3)$$

Because of these interesting, differential modifying effects of this surface-active agent on the action of inhibitors on the enzyme, a series of studies has been carried out to determine modifications in other catalytic properties of glucose-6-*P* phosphohydrolase (Reaction 1), PP_i–glucose phosphotransferase (Reaction 2), and inorganic pyrophosphatase (Reaction 3) activities of the enzyme produced by various concentrations of CTAB. Results of some of these studies with the kidney enzyme, in which detergent-effected alterations in pH optima and apparent Michaelis constant values for the various substrates were assessed, are described in this paper.

MATERIALS AND METHODS

Enzymic assays, protein determination, and sources of most chemicals were as described previously[4,6,7]. CTAB was obtained from Distillation Products, Rochester, N.Y., and was prepared as aqueous solutions of appropriate concentrations (0.5–3.0%, w/v) which were adjusted to pH 7.0 with dilute HCl. Male albino rats (150–200 g) were purchased from Sprague–Dawley, Madison, Wisc., and the microsomal fraction was isolated by differential centrifugation[8] from fresh kidney homogenates prepared in 0.25 M sucrose solution. Microsomes were suspended by homogenization in this same sucrose solution and were diluted to 7 ml per g original wet kidney. 15 min before assay for enzymic activity, these microsomal suspensions were supplemented with 0.1 vol. of distilled water or aqueous CTAB solutions of appropriate concentrations. 0.1-ml aliquots of the resulting preparations were utilized per 1.5-ml reaction mixture.

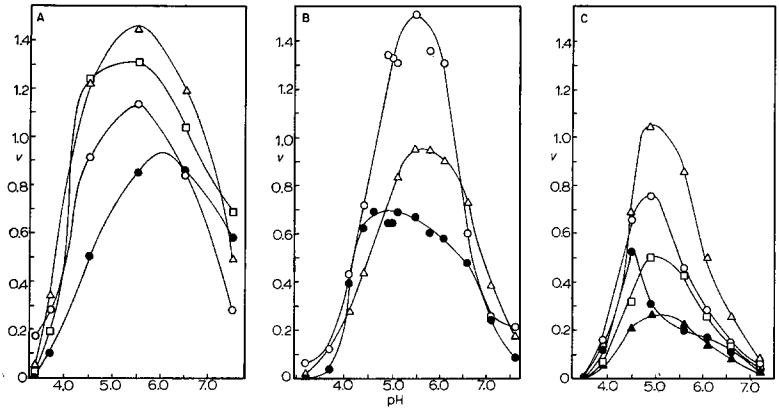

Fig. 1. Effects of various concentrations of CTAB on enzymic activities assayed at a variety of pH values. A. Glucose-6-*P* phosphohydrolase activity. B. Inorganic pyrophosphatase activity. C. PP_i–glucose phosphotransferase activity. Assay mixtures contained, in 1.5 ml, 40 mM sodium cacodylate and 40 mM sodium acetate buffers, 10 mM phosphate substrate (sodium glucose-6-*P* in A; sodium PP_i in B and C), and 0.40 mg microsomal protein. Glucose, 180 mM, was present in C. Kidney microsomal suspensions employed as enzyme source were supplemented with distilled water (●), or with CTAB to final concentrations (w/v) of 0.05 (○), 0.10 (△), 0.20 (□), or 0.30% (▲). Reaction mixtures were prepared in duplicate and pH was measured in one series with a Beckman expanded scale meter, while enzymic activity was assayed in the second series. Velocity, *v*, is expressed in terms of μmoles of substrate hydrolyzed (A or B) or μmoles of glucose-6-*P* formed (C) per 10 min incubation.

Incubations were carried out routinely for 10 min at 30 ± 0.1°, with shaking. Further details are given in legends to individual figures and tables.

RESULTS AND DISCUSSION

Effects of CTAB on pH–activity profiles

The results of experiments in which glucose-6-*P* phosphohydrolase, inorganic pyrophosphatase, and PP_i–glucose phosphotransferase activities were measured as a function of assay mixture pH are described in Figs. 1A, 1B, and 1C, respectively. Water-supplemented microsomal suspensions, and suspensions of microsomes to which various concentrations of CTAB had been added, were employed as enzyme source. With each activity, the detergent produced concentration-dependent alterations both in pH optima and in levels of activity demonstrable at various pH values. The presence of 0.10% (w/v) CTAB in microsomal preparations in all cases produced maximal activation, while higher levels of the detergent were less effective and in one case (0.30% CTAB; see Fig. 1C) actually inhibited phosphotransferase activity. The pH optimum for glucose-6-*P* phosphohydrolase activity was shifted towards acidic pH in the presence of detergent (Fig. 1A), whereas the rather acidic pH optima of inorganic pyrophosphatase (Fig. 1B) and PP_i–glucose phosphotransferase (Fig. 1C) activities (approximately pH 4.5 in both cases) were shifted towards neutrality by the various concentrations of detergent studied. It is apparent from these experiments that the effects of CTAB on enzymic activities are rather complex, the extents of activation (or inhibition) being dependent both on the concentration of the detergent and on assay mixture pH.

Kinetic studies

Since alterations in pH–activity profiles may reflect not only changes in overall reaction rates, but also may be related to changes in apparent Michaelis constant values for substrates induced by the detergent, series of kinetic studies of the various activities were carried out at pH 4.5, 5.5, and 6.5. In all cases, K_m and V or V' values* were determined with control (water-supplemented) microsomal preparations and with such preparations which had been supplemented to 0.05, 0.10, 0.20, or 0.30% (w/v) with CTAB. Some representative results of these studies, carried out at pH 5.5, are presented in Fig. 2, A and B, and Fig. 3, A and B, along with specific experimental details. The K_m values were calculated in all cases as negative reciprocals of x-axis intercepts[11] of extrapolations of double-reciprocal plots[12] of data from experiments in which initial reaction velocities were measured as a function of varied concentrations

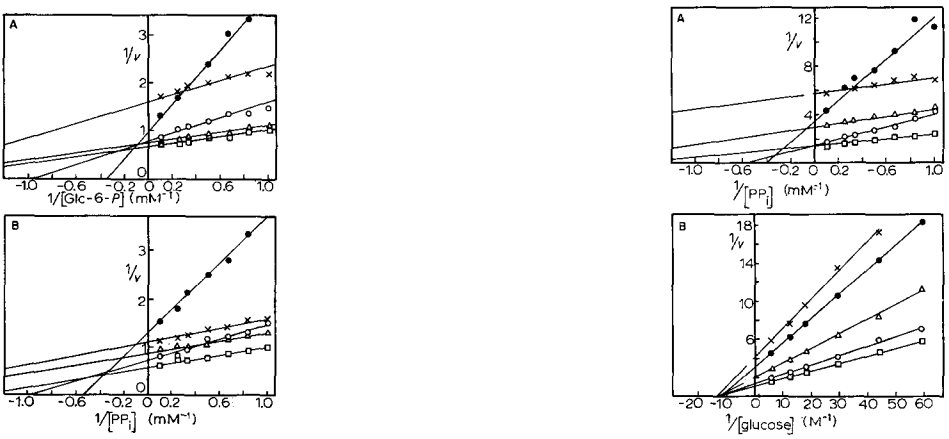

Fig. 2. Effects of CTAB on the kinetics of glucose-6-*P* phosphohydrolase (A) and inorganic pyrophosphatase (B) activity at pH 5.5. Reaction mixtures contained, in 1.5 ml, 40 mM sodium cacodylate and 40 mM sodium acetate buffers, 0.40 mg microsomal protein, and the indicated concentrations of sodium glucose-6-*P* (A), or PP_i (B). Microsomal suspensions were supplemented with CTAB to the following final concentrations (w/v) prior to assay: 0.00, (●); 0.05, (○); 0.10 (□); 0.20, (△); or 0.30%, (×). Activity, v, is expressed as μmoles of substrate hydrolyzed per 10 min. V and K_m values were calculated from these data as described in the text, and are included in Tables I and II.

Fig. 3. Effects of CTAB on the kinetics of PP_i–glucose phosphotransferase activity at pH 5.5. Basic assay mixtures contained, in 1.5 ml, 40 mM sodium cacodylate and 40 mM sodium acetate buffers and 0.40 mg microsomal protein. In (A), glucose was held at 180 mM and sodium PP_i concentration was varied as indicated, while in (B) sodium PP_i was held constant at 10 mM and glucose concentrations were varied. Microsomal suspensions were supplemented with CTAB prior to assay as in Fig. 2. Activity, v, is expressed as μmoles glucose-6-*P* formed per 10 min. V' and K_m values, calculated as described in the text, are included in Table III.

* The following symbols and definitions are employed for kinetic parameters: K_m denotes apparent Michaelis constant; V indicates apparent maximal reaction velocity for glycose-6-*P* phosphohydrolase and inorganic pyrophosphatase reactions; and V' indicates apparent maximal reaction velocity values determined in studies of PP_i–glucose phosphotransferase activity in which either glucose or PP_i concentration was held constant and activity was extrapolated for infinite concentration of either PP_i or glucose, respectively. The use of the term apparent with all parameters is necessitated by the fact that such values vary with detergent concentration. Michaelis constant values for PP_i and glucose in the phosphotransferase reaction previously[9,10] have been shown to be independent of concentration of second substrate.

TABLE I

APPARENT MICHAELIS CONSTANT AND MAXIMAL REACTION VELOCITY VALUES FOR GLUCOSE-6-P PHOSPHOHYDROLASE AT VARIOUS CTAB CONCENTRATIONS AND REACTION pH VALUES

CTAB concentrations (w/v) in microsomal suspensions. V values are expressed as μmoles glucose-6-*P* hydrolyzed per 10 min per 1.5 ml reaction mixture.

CTAB concn. (%, w/v)	K_m *(mM)*			V		
	pH 4.5	*pH 5.5*	*pH 6.5*	*pH 4.5*	*pH 5.5*	*pH 6.5*
0.00	2.9	2.8	2.7	0.67	1.00	1.06
0.05	1.0	1.0	1.1	1.27	1.25	1.14
0.10	0.48	0.56	0.72	1.33	1.49	1.22
0.20	0.29	0.50	0.67	0.87	1.33	0.89
0.30	0.22	0.36	0.50	0.76	0.61	0.67

of substrate. Phosphate substrate concentrations were in all instances varied between 1.0 and 10 mM. Glucose concentrations studied in the phosphotransferase reaction were varied between 18.0 and 180 mM in the presence of 10 mM PP_i, and 180 mM glucose was present when PP_i concentrations were varied. V or V' values were calculated as the y-axis intercepts of these same plots[11]. Kinetic parameters thus calculated for glucose-6-*P* phosphohydrolase, inorganic pyrophosphatase, and PP_i–glucose phosphotransferase at the various assay pH values are presented in Tables I, II, and III, respectively.

It is clear from data in Figs. 2 and 3 and Tables I–III that, at all pH values studied, the various concentrations of CTAB alter quite significantly both K_m and V or V' values of all three enzymic activities. On the basis of extrapolated V and V' values as with v data (Figs. 1A–1C), maximal stimulation of all activities was noted with 0.10% CTAB. Higher concentrations of the detergent were less effective in this respect, and in certain cases (0.20 and 0.30% CTAB with glucose-6-*P* phosphohydrolase (Table I) and PP_i–glucose phosphotransferase (Table III) at pH 6.5; 0.30% CTAB with both these same activities at pH 5.5) actually reduced activity to below-control (CTAB absent) levels.

TABLE II

APPARENT MICHAELIS CONSTANT AND MAXIMAL REACTION VELOCITY VALUES FOR INORGANIC PYROPHOSPHATASE AT VARIOUS CTAB CONCENTRATIONS AND REACTION pH VALUES

CTAB concentrations (w/v) in microsomal suspensions. V values are expressed as μmoles PP hydrolyzed per 10 min per 1.5 ml reaction mixture.

CTAB concn. (%, w/v)	K_m *(mM)*			V		
	pH 4.5	*pH 5.5*	*pH 6.5*	*pH 4.5*	*pH 5.5*	*pH 6.5*
0.00	1.2	1.8	1.4	0.77	0.77	0.58
0.05	0.59	1.1	1.2	1.2	1.66	1.11
0.10	—*	0.87	0.91	—*	2.00	1.40
0.20	—*	0.48	0.57	—*	1.13	0.91
0.30	—*	0.37	0.43	—*	0.92	0.71

* Values could not be determined due to substrate inhibition with these concentrations of CTAB at pH 4.5.

TABLE III

APPARENT MICHAELIS CONSTANT AND MAXIMAL REACTION VELOCITY VALUES FOR PP_i–GLUCOSE PHOSPHOTRANSFERASE AT VARIOUS CTAB CONCENTRATIONS AND REACTION pH VALUES

CTAB concentrations (w/v) in microsomal suspensions. V' values are expressed as μmoles of glucose-6-*P*.

CTAB concn. (%, *w/v*)	K_m (*mM*)			V'		
	pH 4.5	*pH 5.5*	*pH 6.5*	*pH 4.5*	*pH 5.5*	*pH 6.5*
Series I. PP_i concn. varied						
0.00	2.4	2.4	3.0	0.70	0.28	0.26
0.05	1.1	1.7	2.0	1.04	0.70	0.32
0.10	—*	0.83	1.2	—*	0.76	0.38
0.20	—*	0.53	0.63	—*	0.34	0.15
0.30	—*	0.33	0.50	—*	0.18	0.08
Series II. Glucose concn. varied						
0.00	130	83	120	1.11	0.33	0.29
0.05	67	74	80	1.11	0.77	0.39
0.10	67	74	80	1.11	0.91	0.54
0.20	80	74	120	0.61	0.46	0.21
0.30	95	74	120	0.38	0.24	0.14

* Values could not be determined due to substrate inhibition with these concentrations of CTAB at pH 4.5.

In marked contrast with these biphasic effects of the detergent on V or V' values, a reduction in K_m values for all phosphate substrates progressive with increasing concentrations of CTAB was noted with the three activities at all pH values studied. The K_m values for glucose, in contrast, were only modestly altered by the detergent (see Table III). In earlier studies with the liver enzyme[13,14], this K_m value was likewise found relatively refractory to the detergent sodium deoxycholate. The K_m values for PP_i obtained with 0.30% CTAB—0.3 to 0.4 mM (see Tables II and III)—are the lowest values yet reported for this parameter; possibly actual values *in vivo* are even lower, and may be approximated *in vitro* only when ideal sets of experimental conditions can be attained.

It is apparent from data in Figs. 1A–1C and in Tables I–III that CTAB can, in a concentration-dependent manner, produce either an increase or a decrease in enzymic activity values. Such observations suggest that the detergent may function

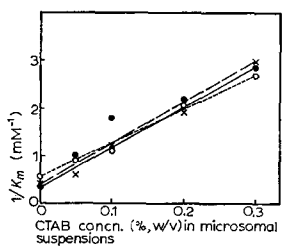

Fig. 4. Variation of 1/(apparent Michaelis constant) values with CTAB concentrations. Data, from Tables I–III (pH 5.5), are plotted by the method suggested by FRIEDENWALD AND MAENGWYN-DAVIES[15]. Data for glucose-6-*P* phosphohydrolase (●), inorganic pyrophosphatase (○), and PP_i–glucose phosphotransferase (×) are described.

both as an activator at lower concentrations and as an inhibitor of the system when present at higher concentrations. The results of analysis of data in Tables I–III according to the graphical method of FRIEDENWALD AND MAENGWYN-DAVIES[15] also support this idea. Reasonably linear plots were obtained when reciprocals of apparent Michaelis constant values from the tables were plotted as a function of CTAB concentrations (see Fig. 4). According to FRIEDENWALD AND MAENGWYN-DAVIES[15], such a linear inverse relationship between K_m values and effector concentrations should exist when a compound (in this case CTAB) produces both "coupling" activation and noncompetitive inhibition of an enzyme system. Coupling activation is defined[15] to involve the promotion by the activator of increased association of enzyme with substrate, and is intuitively apparent from the progressive diminution in K_m values with elevations of CTAB noted even when V or V' values are reduced to sub-control levels by this detergent (see Tables I–III).

General considerations

It is apparent from the present studies that CTAB (a) increases markedly the affinity of the enzyme for phosphate substrates, as revealed by the progressive reduction in K_m values for these compounds observed with increasing concentrations of the detergent (see Tables I–III); (b) exerts an additional effect in accelerating the overall rates of reactions above and beyond that due to reduction of K_m values, as is apparent from the increases in V and V' (Tables I–III) which are extrapolated to infinite concentrations of phosphate substrates or glucose; and (c) inhibits the activated enzyme in a kinetically noncompetitive fashion at higher concentrations[15].

It appears likely that these effects of the detergent on kinetic properties of the enzyme may be closely related to the fact that glucose-6-phosphatase is either an integral part of, or very tightly bound to, the phospholipid-rich, hydrophobic membrane of the endoplasmic reticulum[16]. Factors capable of affecting this membrane—for example, phospholipase treatment and phospholipid supplementation[17,18] and natural detergents such as long-chain fatty acyl-CoA esters[19]—dramatically alter catalytic properties of both phosphohydrolase and phosphotransferase activities of this enzyme. CTAB previously has been observed to induce alterations in catalytic properties of certain other membrane-bound enzymes[20–22]. Rather marked differences in catalytic properties of a number of enzymes have been noted when the catalysts were embedded in synthetic membranes or attached to subcellular particles (see refs. 23 and 24). Gross changes in morphology of the membrane of endoplasmic reticulum produced by CTAB could indirectly result in an increased accessibility of the enzyme's active site to phosphate substrates, leading to the noted reductions in K_m values for these substrates. Alternatively, the charge on the microsomal membrane might be made more positive as a result of the adsorption of the cationic detergent CTAB, which in turn would lead to a relative increase in the local concentration of negatively-charged phosphate substrates thus causing a decrease in apparent Michaelis constant values for these substrates (but not for uncharged glucose). Thirdly, it also is possible that conformational alterations in the enzyme, either resulting indirectly from detergent-produced changes in the membrane of the endoplasmic reticulum or produced by direct action of the detergent on the enzyme molecule, may be involved in the noted alterations both in affinity (K_m) and velocity (v, V and V') terms associated with the various activities of the enzyme. A more detailed mechanistic consideration of these

interesting effects of CTAB must await the availability of this enzyme in highly purified form.

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REFERENCES

1 R. C. NORDLIE, T. L. HANSON, P. T. JOHNS AND D. G. LYGRE, *Proc. Natl. Acad. Sci. U.S.*, 60 (1968) 590.
2 A. L. VIANNA AND R. C. NORDLIE, *J. Biol. Chem.*, 244 (1969) 4027.
3 R. C. NORDLIE, *Ann. N.Y. Acad. Sci.*, in the press.
4 J. F. SOODSMA, B. LEGLER AND R. C. NORDLIE, *J. Biol. Chem.*, 242 (1967) 1955.
5 D. G. LYGRE AND R. C. NORDLIE, *Biochim. Biophys. Acta*, 185 (1969) 360.
6 R. C. NORDLIE AND W. J. ARION, *J. Biol. Chem.*, 239 (1964) 1680.
7 R. C. NORDLIE AND W. J. ARION, *Methods Enzymol.*, 9 (1966) 619.
8 C. DE DUVE AND J. BERTHET, *Intern. Rev. Cytol.*, 3 (1954) 225.
9 W. J. ARION AND R. C. NORDLIE, *J. Biol. Chem.*, 239 (1964) 2752.
10 R. C. NORDLIE AND J. F. SOODSMA, *J. Biol. Chem.*, 241 (1966) 1719.
11 M. DIXON AND E. C. WEBB, *Enzymes*, Academic Press, New York, 1964, p. 67.
12 H. LINEWEAVER AND D. BURK, *J. Am. Chem. Soc.*, 56 (1934) 658.
13 R. C. NORDLIE, W. J. ARION AND E. A. GLENDE, JR., *J. Biol. Chem.*, 240 (1965) 3479.
14 R. C. NORDLIE, W. J. ARION, T. L. HANSON, J. R. GILSDORF AND R. N. HORNE, *J. Biol. Chem.*, 243 (1968) 1140.
15 J. S. FRIEDENWALD AND G. D. MAENGWYN-DAVIES, in W. D. MCELROY AND B. GLASS, *The Mechanism of Enzyme Action*, Johns Hopkins Press, Baltimore, 1954, p. 180.
16 L. ERNSTER, P. SIEKEVITZ AND G. E. PALADE, *J. Cell Biol.*, 15 (1962) 541.
17 S. M. DUTTERA, W. L. BYRNE AND M. C. GANOZA, *J. Biol. Chem.*, 243 (1968) 2216.
18 R. E. SNOKE AND R. C. NORDLIE, *Abstr. Natl. Meeting Am. Chem. Soc., 156th, Atlantic City, N.J., 1968*, Abstract BIOL. 102.
19 R. C. NORDLIE, T. L. HANSON AND P. T. JOHNS, *J. Biol. Chem.*, 242 (1967) 4144.
20 C. B. TAYLOR, *J. Physiol. London*, 165 (1963) 199.
21 P. D. SWANSON, H. F. BRADFORD AND H. MCILWAIN, *Biochem. J.*, 92 (1964) 235.
22 H. F. BRADFORD, P. D. SWANSON AND D. B. GAMACK, *Biochem. J.*, 92 (1964) 247.
23 I. H. SILMAN AND E. KATCHALSKI, *Ann. Rev. Biochem.*, 35 (1966) 873.
24 E. KATCHALSKI, *Intern. Congr. Biochem., Tokyo, 1967*, Abstr. Vol. I, p. 147.