Biochem. J. (1981) **193**, 709–716
Printed in Great Britain

Properties of rat renal phosphate-dependent glutaminase coupled to Sepharose

Evidence that dimerization is essential for activation

Roberta F. MOREHOUSE and Norman P. CURTHOYS
Department of Biochemistry, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261, U.S.A.

(*Received 28 July 1980/Accepted 13 October 1980*)

In the absence of phosphate, purified rat renal phosphate-dependent glutaminase exists as a catalytically inactive protomer. The addition of phosphate results in both dimerization and activation of the glutaminase. Covalent attachment of the dimeric form of the glutaminase to CNBr-activated Sepharose was achieved with 84% retention of activity. At least 70% of the bound glutaminase activity was expressed even in the absence of added phosphate. In addition, 6-diazo-5-oxo-L-norleucine, which interacts only with the catalytically active form of the glutaminase, inactivates the bound dimeric form of glutaminase at the same rate in either the absence or the presence of added phosphate. Therefore retention of dimeric structure is apparently sufficient to maintain glutaminase activity. In contrast, the coupling of the protomeric form of the enzyme to Sepharose resulted in retention of only 3% of the phosphate-induced glutaminase activity. However, up to 48% of this activity could be reconstituted by addition of soluble glutaminase under conditions that promote dimerization. These results indicate that the monomeric form of the glutaminase has minimal inherent activity and that dimerization is an essential step in the phosphate-induced activation of the glutaminase.

As part of the homoeostatic adaptation to metabolic acidosis, the kidney exhibits an increased production and excretion of NH_4^+ ions (Pitts, 1973; Tannen, 1978). Increased renal ammoniagenesis provides an expendable cation, which facilitates the excretion of anions or titratable acids without depleting the reserves of Na^+ and K^+. NH_4^+ ions produced in the kidney are derived from glutamine, which is extracted from the plasma (Van Slyke *et al.*, 1943; Squires *et al.*, 1976). Renal glutamine metabolism is primarily initiated by a phosphate-dependent glutaminase (Goldstein, 1967; Tannen & Kunin, 1976), which is associated with the mitochondrial inner membrane (Curthoys & Weiss, 1974; Kovačević, 1976).

After freeze-drying of renal mitochondria in the presence of a borate/phosphate/pyrophosphate buffer, the glutaminase is solubilized by autolysis (Clark & Curthoys, 1979). In the presence of the borate-containing buffer, the solubilized glutaminase undergoes aggregation to produce a form of the enzyme with a molecular weight greater than 10^7 (Curthoys *et al.*, 1976). The non-aggregated form of glutaminase exhibits an absolute dependence on phosphate or other multivalent anion for activity. In the absence of phosphate, the glutaminase exists as a catalytically inactive protomer of molecular weight approx. 160000 (Godfrey *et al.*, 1977). Activation of the glutaminase is associated with a phosphate-dependent dimerization, whereas glutamate inhibition causes the reversal of dimer formation. These observations, along with the results of experiments with affinity labels (Shapiro *et al.*, 1978, 1979), have led to the hypothesis that dimer formation may be essential for enzyme activation. However, the results of these studies are also consistent with the possibility that binding of phosphate changes the conformation of the protomer sufficiently to produce activation and that this new conformation subsequently leads to self-association.

The importance of considering this alternative causal sequence is clearly illustrated by the previous characterization of bovine liver glutamate dehydrogenase, which also undergoes a concentration-dependent polymerization that is influenced by various ligands. The close correlation observed between the effects of nicotinamide nucleotides on the association–dissociation and the kinetics of glutamate dehydrogenase led to the initial suggestion that aggregation of this enzyme was essential for its catalytic function (Frieden, 1959). However, when changes in molecular weight and specific activity were compared under identical conditions, it became

0306-3275/81/030709-08$01.50/1

apparent that the associated and dissociated forms of the dehydrogenase exhibit identical activities (Fisher *et al.*, 1962). It was subsequently shown (Frieden, 1963) that compounds that cause dissociation at high concentrations of enzyme result in the formation of a structurally altered subunit, which is essentially inactive and which can no longer undergo association. In the present study we have characterized the properties of the protomeric and dimeric forms of the glutaminase immobilized on CNBr-activated Sepharose and have obtained evidence that establishes that dimerization is essential for phosphate-induced activation of the glutaminase.

Experimental

Materials

White male Sprague–Dawley rats (200–300g) were obtained from Zivic Miller (Allison Park, PA, U.S.A.) and were maintained on Purina rat chow. Glutamate dehydrogenase was purchased from Boehringer (New York, NY, U.S.A.). Sepharose 4B was obtained from Pharmacia (Piscataway, NJ, U.S.A.). CNBr was purchased from Eastman Co. (Rochester, NY, U.S.A.). 6-Diazo-5-oxo-L-norleucine was synthesized by Richard A. Shapiro, using methods previously reported (Holcenberg *et al.*, 1978; Shapiro *et al.*, 1979). All other biochemicals were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Glutaminase preparation and assay

Phosphate-dependent glutaminase was purified from kidneys of acidotic rats as described previously (Curthoys *et al.*, 1976). Purified glutaminase preparations were stored at 1–2mg/ml in 10mM-sodium borate / 100mM-potassium phosphate / 100mM-potassium pyrophosphate buffer, pH8.9, at 4°C. Before use in various experiments, samples of the glutaminase were dialysed for 4h against two 1-litre volumes of 10mM-sodium barbital buffer, pH8.6, with or without the addition of 150mM-potassium phosphate. Unless otherwise indicated, all glutaminase assays were performed in the presence of 150mM-potassium phosphate (Curthoys & Weiss, 1974). In order to determine the glutaminase activity covalently bound to Sepharose, samples were diluted at least 4-fold with buffer and gently vortex-mixed to produce a slurry. Samples (25–100μl) of the slurry were mixed with 100μl of a 2-fold-concentrated assay mixture diluted with sufficient water to produce a final volume of 200μl. Constant gentle agitation was required during the incubation at 37°C in order to obtain linear and reproducible assays. Glutaminase activity was terminated by heating the sample to 90°C for 2min. The glutamate formation was determined by using glutamate dehydrogenase (Curthoys & Lowry, 1973). A unit of glutaminase activity is the amount of enzyme that produces 1μmol of glutamate/min at 37°C. For inactivation experiments, 100μl samples containing glutaminase coupled to Sepharose and the desired concentrations of 6-diazo-5-oxo-L-norleucine and phosphate were incubated at 20°C. After various times, separate samples were removed and used to determine the remaining glutaminase activity. Protein concentration was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as the standard.

CNBr activation of Sepharose 4B

Sepharose 4B equilibrated with 10mM-sodium barbital buffer, pH8.6, was adjusted to pH11.0 just before addition of CNBr (25 or 60mg/ml of Sepharose) dissolved in 1ml of acetonitrile. During the activation reaction, the Sepharose slurry was stirred constantly, the temperature was maintained at 20°C, and the pH was maintained at 11 (Cuatrecasas, 1970). After 10min, the reaction was terminated by throughly washing the Sepharose with cold barbital buffer, pH8.6. When the Sepharose was to be used for attaching the dimeric form of the glutaminase, the buffer also contained 150mM-potassium phosphate.

Coupling of glutaminase to Sepharose 4B

Phosphate-dependent glutaminase, dialysed against 10mM-sodium barbital buffer, pH8.6, with or without 150mM-phosphate, was mixed with activated Sepharose in a ratio of 80–160μg of protein/ml of Sepharose and incubated at 4°C for 2h without further agitation. The Sepharose was allowed to settle, and the supernatant was removed and assayed for glutaminase activity. The Sepharose was then washed and incubated overnight at 4°C with buffer containing 125mM primary amine in order to block the remaining CNBr-activated sites. The Sepharose was then washed thoroughly and assayed for glutaminase activity.

As a control for glutaminase inactivation and non-specific binding to the Sepharose, a portion of non-activated Sepharose was also incubated with glutaminase. After the 2h incubation, the Sepharose was allowed to settle, and a sample of the supernatant was assayed for glutaminase activity. The primary amine used as a blocking agent was then added as a concentrated solution to the resuspended slurry. After the overnight incubation with the blocking agent, the Sepharose was separated from the supernatant and washed with cold buffer. Both were then assayed for glutaminase activity.

Reconstitution of glutaminase bound to Sepharose 4B

A 1ml sample of Sepharose containing phos-

phate-dependent glutaminase coupled in the absence of phosphate (protomeric form) was packed into a small column and equilibrated with 10mM-sodium barbital / 150mM-potassium phosphate buffer, pH8.6. Glutaminase in barbital/phosphate buffer (dimeric form) was added to the column and incubated for 15min at 4°C. The Sepharose column was then washed with several 1ml batches of buffer. Each of the eluates and the resuspended Sepharose were then assayed for glutaminase activity. As a control for non-specific binding of glutaminase, a sample of CNBr-activated Sepharose that had been blocked with a primary amine was incubated with glutaminase and washed in the same manner.

Results

Coupling in the presence of phosphate

The initial coupling experiments were performed under conditions where the glutaminase was known to be present as a dimer. When incubated with CNBr-activated Sepharose in the presence of 150mM-potassium phosphate (Table 1), 84% of the added glutaminase activity became associated with the gel. Less than 1% of the added glutaminase activity was recovered in the supernatant fractions. The bound glutaminase activity was unaffected by subsequent washing with barbital or barbital/phosphate buffers. When the glutaminase was incubated with the non-activated control gel in the presence of phosphate, only 1% of the added glutaminase activity remained associated with the gel, but 69% of the activity was recovered in the supernatant fractions.

As shown in Fig. 1, the phosphate activation profile of the glutaminase bound to Sepharose in the presence of phosphate is significantly different from that of the unbound glutaminase. The activity of the soluble glutaminase exhibits an absolute dependence on the presence of phosphate, with half-maximal saturation at 25mM (Shapiro *et al.*, 1978). In contrast, the bound glutaminase, even though washed extensively with barbital buffer in the absence of phosphate, retained approx. 70% of its activity when assayed in the absence of phosphate. The small decrease in activity observed in the presence of less than 10mM-potassium phosphate indicated that most of the glutaminase, when coupled as dimers, was bound in such a way that it retained activity even in the absence of added phosphate.

Inactivation of the soluble glutaminase with the affinity label 6-diazo-5-oxo-L-norleucine exhibits an absolute dependence on the presence of phosphate (Shapiro *et al.*, 1979). In contrast, the presence or absence of added phosphate had no effect on the rate of diazoketone inactivation of the Sepharose-bound glutaminase (Fig. 2). This also indicates that the coupling of the glutaminase to Sepharose in the presence of phosphate restricts the structure of the enzyme to its active conformation.

Table 1. *Coupling of phosphate-dependent glutaminase to CNBr-activated Sepharose in the presence of 150mM-potassium phosphate*

A 1ml portion of Sepharose 4B was activated with 60mg of CNBr, equilibrated with 10mM-sodium barbital / 150mM-potassium phosphate buffer, pH8.6, and incubated with 80μg of glutaminase that had been dialysed against barbital/phosphate buffer. After incubation at 4°C for 2h, the CNBr-activated sites that had not reacted were blocked by the addition of 125mM-glycine. As a control of non-specific adsorption during the coupling experiment, an equivalent amount of glutaminase was incubated with non-activated Sepharose. The numbers in parentheses represent the percentage of added glutaminase recovered in each fraction.

	Glutaminase activity (μmol/min)	
	Activated sample gel	Non-activated control gel
Added for binding	9.6 (100)	9.6 (100)
After coupling reaction		
In supernatant	0.3 (0.3)	9.3 (97)
After blocking reaction		
In supernatant	—	6.6 (69)
On gel	8.1 (84)	0.06 (0.6)

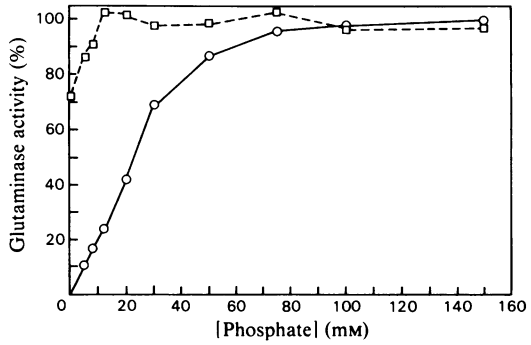

Fig. 1. *Comparison of the effect of phosphate concentration on the activity of the soluble phosphate-dependent glutaminase and the glutaminase coupled to Sepharose in the presence of 150mM-potassium phosphate*

The unbound (○) and the bound (□) glutaminases were equilibrated with 10mM-sodium barbital buffer, pH8.6, by extensive dialysis or washing respectively. Glutaminase activity was determined by the standard assay procedure, except that the phosphate concentration was varied as indicated. Activity is expressed as a percentage of the maximal activity observed at 150mM-phosphate.

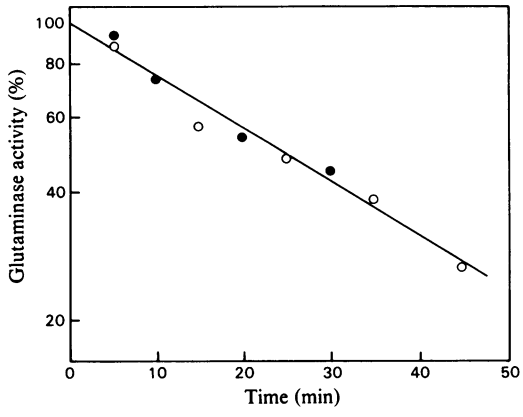

Fig. 2. *Effect of phosphate on the rate of 6-diazo-5-oxo-L-norleucine inactivation of the phosphate-dependent glutaminase coupled to Sepharose in the presence of 150 mM-potassium phosphate*

The Sepharose containing bound glutaminase was washed extensively with 10mM-sodium barbital buffer, pH8.6. The Sepharose slurry was incubated at 20°C with 2.5mM-diazoketone in the absence (○) or in the presence (●) of 100mM-potassium phosphate and then assayed for glutaminase activity at various times as described in the Experimental section. Activity is expressed as a percentage of initial activity present at zero time.

Table 2. *Coupling of phosphate-dependent glutaminase to CNBr-activated Sepharose in the absence of phosphate*

A 1ml portion of Sepharose 4B was activated with 25mg of CNBr, equilibrated with 10mM-sodium barbital buffer, pH8.6, and incubated with 80μg of glutaminase that had been dialysed against barbital buffer. The excess CNBr-activated sites were blocked by the addition of 125mM-glycine. The numbers in parentheses represent the percentage of added glutaminase recovered in each fraction.

	Glutaminase activity (μmol/min)	
	Activated sample gel	Non-activated control gel
Added for binding	9.0 (100)	9.0 (100)
After coupling reaction		
In supernatant	0.9 (10)	8.4 (93)
After blocking reaction		
In supernatant	—	7.3 (81)
On gel	0.23 (3)	0.03 (0.3)

Coupling in the absence of phosphate

Coupling experiments were also performed under conditions where the glutaminase was known to be present as protomers. As shown in Table 2, coupling in the absence of phosphate results in apparent inactivation of the glutaminase. Only 10% of the added activity was recovered in the supernatant fractions and only 3% remained associated with the Sepharose. Analysis of the supernatant fraction obtained after the coupling reaction routinely detected 10% or less of the added protein, indicating that greater than 90% of the glutaminase was bound to the Sepharose as an inactive molecule. In contrast, 81% of the glutaminase activity incubated with the non-activated control gel was recovered in the supernatant fractions and less than 1% remained associated with the Sepharose. The percentage of the glutaminase activity unaccounted for in the control experiment was considerably less than the loss of activity observed when the glutaminase was incubated with the CNBr-activated Sepharose. Therefore the attachment of the protomeric form of the glutaminase to Sepharose results in a preparation that is inactive even when assayed in the presence of phosphate. In order to eliminate the possibility that inactivation results solely from a restriction in the ability of the enzyme to undergo phosphate-induced conformational changes, reconstitution of activity by the subsequent addition of soluble glutaminase was performed.

Reconstitution

Reconstitution procedures were initially designed to promote association of the soluble and bound glutaminase protomers, while minimizing non-specific absorption of the soluble glutaminase to the Sepharose. The previous characterization of the effect of phosphate on the sedimentation coefficient of the glutaminase established that the protomeric and dimeric forms of the glutaminase are in rapid equilibrium (Godfrey *et al.*, 1977). Therefore extensive washing of a column containing reconstituted glutaminase was avoided in order to prevent dissociation. To minimize non-specific adsorption, the amount of glutaminase activity added during the reconstitution was kept approximately equal to the amount of activity initially coupled to the sample gel.

The excess CNBr-activated sites of the Sepharose containing bound glutaminase were blocked by incubation with a primary amine. The structure of the amine used as a blocking agent has a dramatic effect on the non-specific binding of glutaminase dimers. Use of aspartate and glycine resulted in only 1% non-specific adsorption of glutaminase activity, whereas glucosamine resulted in 15% non-specific adsorption. In contrast, the methylamine-blocked gel retained 75% of the glutaminase with which it was initially incubated. Subsequent elution of the methylamine-blocked Sepharose with a barbital buffer containing 1% Triton X-100 did not release any of the adsorbed glutaminase. Because of the effect of

Table 3. *Reconstitution of phosphate-dependent glutaminase coupled to Sepharose in the absence of phosphate*
(*a*) A 1 ml portion of Sepharose 4B was activated with 25 mg of CNBr, equilibrated with 10 mM-sodium barbital buffer, pH 8.6, and incubated with 80 μg of glutaminase that had been dialysed against barbital buffer. The excess CNBr-activated sites were blocked by the addition of 125 mM-glycine. (*b*) A 1 ml column of Sepharose containing bound glutaminase and a 1 ml column of CNBr-activated Sepharose blocked with glycine were equilibrated with barbital buffer containing 150 mM-potassium phosphate and then incubated with 12.6 units of glutaminase. The columns were washed with four 1 ml volumes of the barbital/phosphate buffer and three 1 ml volumes of the barbital buffer. The numbers in parentheses represent the percentage of added glutaminase recovered in each fraction.

	Glutaminase activity (μmol/min)	
(*a*) Coupling (minus phosphate)	Sample gel	
Added for binding	11.2 (100)	
Recovered in supernatant	4.5 (40)	
Recovered on gel	0.3 (3)	
	Glutaminase activity (μmol/min)	
(*b*) Reconstitution (plus phosphate)	Sample gel	Control gel
Added for reconstitution	12.6 (100)	12.6 (100)
Recovered in washes	9.5 (75)	9.0 (71)
Recovered on gel	2.6 (20)	0.36 (3)

the blocking agent on non-specific adsorption of the glutaminase, a sample of CNBr-activated Sepharose that had been treated with the appropriate primary amine was used as a control for the reconstitution experiment.

The results of a reconstitution experiment in which glycine was used as a blocking agent are shown in Table 3. In this experiment, 40% of the glutaminase activity added during the coupling procedure was recovered in the initial supernatant fractions. In spite of the incomplete coupling, the amount of glutaminase activity associated with the reconstituted Sepharose sample was 7-fold greater than that associated with the glycine-blocked control gel. The elution of glutaminase activity from the reconstituted glutaminase-bound Sepharose was retarded in comparison with the elution profile observed for the glycine-blocked control gel (Fig. 3). The recoveries of glutaminase activity in the first two fractions eluted from the control gel were nearly equivalent, and elution was complete with 3 column volumes of barbital/phosphate buffer. In contrast, a large amount of glutaminase activity was eluted only in the second fraction collected from the Sepharose sample containing bound glutaminase. In addition, significant glutaminase activity was released in all subsequent fractions. Only the reconstituted Sepharose sample exhibited a further release of glutaminase activity when washed with barbital buffer in the absence of phosphate.

A higher yield of reconstitution was obtained in an experiment in which methylamine was used as the blocking agent (Table 4). The retention of a greater than usual amount of the added glutaminase activity (17%) after the coupling reaction resulted from the increased ratio of glutaminase to Sepharose (160 μg/ml) used in this experiment. This may reflect a closer proximity of bound protomers on the gel. After

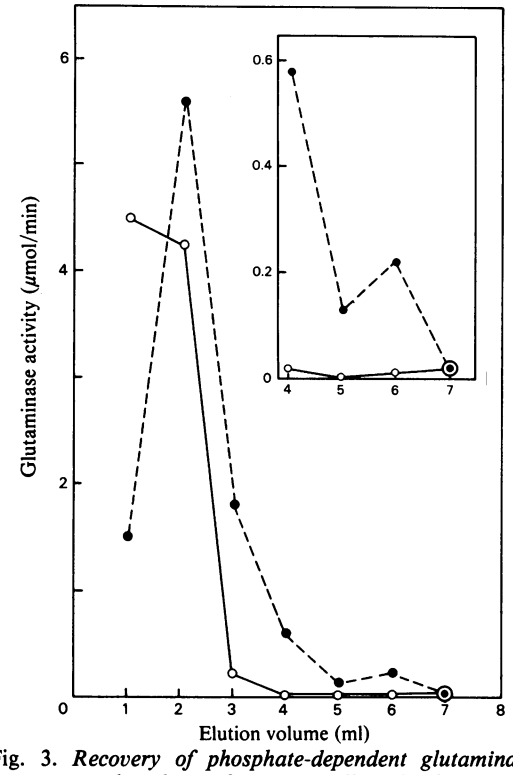

Fig. 3. *Recovery of phosphate-dependent glutaminase activity in the eluate fractions collected after reconstitution of the glutaminase coupled to Sepharose in the absence of phosphate*
A 1 ml column of Sepharose containing bound glutaminase (●) and a 1 ml column of CNBr-activated Sepharose blocked with glycine (○) were incubated with soluble glutaminase and washed with 7 column volumes of buffer as described in Table 3. Each of the column eluates was collected separately and assayed for glutaminase activity. The data for the last four fractions are illustrated with an expanded scale in the insert.

Table 4. *Effect of blocking with methylamine on the reconstitution of phosphate-dependent glutaminase coupled to Sepharose in the absence of phosphate*

(*a*) A 1 ml portion of Sepharose 4B was activated with 25 mg of CNBr, equilibrated with 10 mM-sodium barbital buffer, pH 8.6, and incubated with 160 μg of glutaminase that had been dialysed against barbital buffer. The excess CNBr-activated sites were blocked by the addition of 125 mM-methylamine. (*b*) For reconstitution, a 1 ml column of the Sepharose containing bound glutaminase and a 1 ml column of CNBr-activated Sepharose blocked with methylamine were equilibrated with barbital buffer containing 150 mM-potassium phosphate and then incubated with 12.5 units of glutaminase. Both gels were washed with 4 ml of the barbital/phosphate buffer, but showed no detectable release of glutaminase activity. The numbers in parentheses represent the percentage of added glutaminase recovered in each fraction.

	Glutaminase activity (μmol/min)	
(*a*) Coupling (minus phosphate)	Sample gel	
Added for binding	12.5 (100)	
Recovered in supernatant	0.01 (0.1)	
Recovered on gel	2.1 (17)	
	Glutaminase activity (μmol/min)	
(*b*) Reconstitution (plus phosphate)	Sample gel	Control gel
Added for reconstitution	12.5 (100)	12.5 (100)
Recovered in washes	0.01 (0.1)	0.01 (0.1)
Recovered on gel	19.6 (157)	9.4 (75)

reconstitution with soluble glutaminase, none of the fractions collected during washing of either the reconstituted Sepharose or the methylamine-blocked control gel contained a detectable amount of glutaminase activity. The difference between the activity associated with the reconstituted Sepharose sample (19.6 μmol/min) and the sum of the glutaminase activity added during the reconstitution (12.5 μmol/min) and that retained after the initial coupling reaction (2.1 μmol/min) was 5.0 μmol/min. A comparison of this difference with the maximal amount of the glutaminase initially bound to CNBr-activated Sepharose as inactive protomers (10.4 μmol/min) indicates that a minimum of 48% of the glutaminase was reconstituted. Apparently the methylamine also serves to stabilize the reconstituted glutaminase and prevents its subsequent dissociation.

Discussion

Phosphate-dependent glutaminase, coupled to Sepharose in the presence of phosphate, retained about 85% of its original activity. Therefore chemical modification of its amino residues or steric hindrance due to the proximity of the Sepharose bead have only minor effects on the activity of the glutaminase when bound as dimers. The glutaminase activity was not released when the Sepharose containing covalently bound glutaminase was washed thoroughly with buffer in the absence of phosphate. Thus most of the glutaminase when coupled under these conditions is apparently bound to the Sepharose through both protomers. In addition, only 30% of the Sepharose-bound glutaminase activity was dependent on phosphate. The glutaminase coupled as dimers also showed the same rate of inactivation by 6-diazo-5-oxo-L-norleucine when incubated in either the absence or the presence of 100 mM-potassium phosphate. These results suggest that retention of the dimeric structure may be sufficient to maintain glutaminase activity even in the absence of phosphate. However, the experiments presented here cannot eliminate the possibility that coupling of the glutaminase to Sepharose restricts the structure of the enzyme so that phosphate remains tightly bound to the coupled enzyme.

Phosphate-dependent glutaminase coupled to CNBr-activated Sepharose in the absence of phosphate retained only 3% of its original activity. Since the protomeric form of the glutaminase is less stable (Godfrey *et al.*, 1977), chemical modification could contribute to its inactivation. Alternatively, the covalent attachment of the protomer to Sepharose could prevent phosphate-induced changes in conformation, which are essential for activation. However, the use of similar procedures to attach *Escherichia coli* carbamoyl phosphate synthetase to Sepharose had little effect on its ability to undergo conformational changes (Anderson, 1977). Oligomerization of the synthetase is promoted by ATP and by positive allosteric effectors, but is reversed by negative effectors. Monomers of the synthetase when covalently bound to Sepharose retained 80% of their original activity and exhibited a normal response to

allosteric effectors. If chemical modification or restricted conformation were responsible for its apparent inactivation, the bound protomeric form of the glutaminase would not undergo reconstitution and re-activation after addition of soluble glutaminase. Since reconstitution experiments resulted in re-activation of at least 48% of the bound glutaminase, the inherent activity of the protomeric form of the enzyme must be minimal, and phosphate-induced conformational changes cannot be sufficient to cause activation.

Because of the limitations of the methods used, the achievement of 100% reconstitution is unlikely. Reconstitution requires the presence of a rapid equilibrium between the protomeric and dimeric forms of glutaminase. However, the reversibility of this equilibrium would also make possible the dissociation of reconstituted dimers. The elution profiles of glutaminase activity observed in the reconstitution experiments in which glycine was used to block the excess CNBr-activated sites indicated that even the minimal washing procedure used caused dissociation of the reconstituted glutaminase (Fig. 3). The glutaminase bound to Sepharose, which was subsequently blocked with methylamine, exhibited a high percentage of reconstitution. Apparently the ability of the Sepharose containing covalently bound methylamine to adsorb the glutaminase strongly also contributes stability to the reconstituted enzyme.

Dimerization of glutaminase protomers must occur through specific interactions. The glutaminase protomers are probably bound to Sepharose through multiple sites and without regard to orientation. Therefore a portion of the protomers are probably bound to the Sepharose in such a way that the specific sites required for dimerization either are modified or are held in close proximity to the Sepharose and can no longer interact with a second protomer. The protomeric form of the glutaminase is less stable than the dimeric form. Therefore partial denaturation of the glutaminase protomers could occur either before or after the coupling reaction. Denaturation of bound enzyme subunits has been shown to affect the yield of reconstitution in experiments with lactate dehydrogenase (Chan & Mosbach, 1976). After coupling of lactate dehydrogenase to Sepharose by only one of its four subunits, the tetramer was dissociated to yield Sepharose containing single subunits. The percentage of reconstitution varied from 45 to 78% among different preparations of Sepharose-bound subunits. If the gel were stored for 24h at 4°C before reconstitution, the yield of reconstitution was decreased by 40%.

With consideration of these limitations, the observed extent of reconstitution strongly supports the hypothesis that dimer formation is an essential step in activation of the solubilized glutaminase. In other words, conformational changes produced by protomer–protomer interactions are essential for activation of the glutaminase.

These findings may be important in contributing to our understanding of the regulation of renal ammoniagenesis. After 7 days of chronic acidosis, increased rat renal ammoniagenesis may be facilitated by the 20-fold increase in the concentration of glutaminase that occurs within the proximal convoluted tubules (Curthoys & Lowry, 1973). However, during onset of acute acidosis, increased ammonia production occurs within 1h (Boyd & Goldstein, 1979; Tannen & Ross, 1979) and significantly precedes any increase in the glutaminase concentration. Activation of the glutaminase during this period is probably related to changes in the concentrations of small metabolites such as phosphate and glutamate. Physiological concentrations of glutamate and phosphate significantly affect glutaminase activity (Goldstein, 1966, 1967). Rapid decreases in renal glutamate and α-oxoglutarate concentrations have been reported to occur after onset of acidosis (Boyd & Goldstein, 1979; Vinay *et al.*, 1980). Therefore the regulation *in vivo* of the phosphate-dependent glutaminase may proceed through ligand-induced changes in its extent of dimerization and activation.

The investigation was supported in part by Research Grant AM 16651 and Research Career Development Award AM 00068 (to N. P. C.) from the National Institute of Arthritis, Metabolism and Digestive Diseases.

References

Anderson, P. (1977) *Biochemistry* **16**, 583–586
Boyd, T. A. & Goldstein, L. (1979) *Am. J. Physiol.* **329**, E289–E294
Chan, W. W. C. & Mosbach, K. (1976) *Biochemistry* **15**, 4215–4222
Clark, V. M. & Curthoys, N. P. (1979) *J. Biol. Chem.* **254**, 4939–4941
Cuatrecasas, P. (1970) *J. Biol. Chem.* **245**, 3059–3065
Curthoys, N. P. & Lowry, O. H. (1973) *J. Biol. Chem.* **248**, 162–168
Curthoys, N. P. & Weiss, R. F. (1974) *J. Biol. Chem.* **249**, 3261–3266
Curthoys, N. P., Kuhlenschmidt, T. & Godfrey, S. S. (1976) *Arch. Biochem. Biophys.* **174**, 82–89
Fisher, H. F., Cross, D. G. & McGregor, L. L. (1962) *Nature (London)* **196**, 895–896
Frieden, C. (1959) *J. Biol. Chem.* **234**, 809–814
Frieden, C. (1963) *Biochem. Biophys. Res. Commun.* **10**, 410–415
Holcenberg, H. C., Ericsson, L. & Roberts, J. (1978) *Biochemistry* **17**, 411–417
Godfrey, S., Kuhlenschmidt, T. & Curthoys, N. P. (1977) *J. Biol. Chem.* **252**, 1927–1931
Goldstein, L. (1966) *Am. J. Physiol.* **210**, 661–666
Goldstein, L. (1967) *Am. J. Physiol.* **213**, 983–989

Kovačević, Z. (1976) *Biochim. Biophys. Acta* **430**, 399–412

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275

Pitts, R. F. (1973) *Handb. Physiol. Sect. 8: Renal Physiol.* 445–496

Shapiro, R. A., Clark, V. M. & Curthoys, N. P. (1978) *J. Biol. Chem.* **253**, 7086–7090

Shapiro, R. A., Clark, V. M. & Curthoys, N. P. (1979) *J. Biol. Chem.* **254**, 2835–2838

Squires, E. J., Hall, D. E. & Brosnan, J. T. (1976) *Biochem. J.* **160**, 125–128

Tannen, R. (1978) *Am. J. Physiol.* **235**, F265–F277

Tannen, R. L. & Kunin, A. S. (1976) *Am. J. Physiol.* **231**, 1631–1637

Tannen, R. L. & Ross, B. D. (1979) *Clin. Sci. Mol. Med.* **56**, 353–364

Van Slyke, D. D., Phillips, R. A., Hamilton, P. B., Archibald, R. M., Futcher, P. H. & Hiller, A. (1943) *J. Biol. Chem.* **150**, 481–482

Vinay, P., Allignet, E., Pichette, C., Watford, M., Lemieux, G. & Gougoux, A. (1980) *Kidney Int.* **17**, 312–325