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Free-Energy Changes of the Glutaminase Reaction and the Hydrolysis of the Terminal Pyrophosphate Bond of Adenosine Triphosphate

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In the preceding paper, Kitzinger & Hems (1959) found differences between the heats of hydrolysis of the amide groups of asparagine and glutamine and concluded that it was not reasonable to assume that the free energies of hydrolysis were closely similar. ΔG^0 for the hydrolysis of glutamine could not therefore be estimated from the available value for asparagine. As previously discussed, the interest of free-energy data for the hydrolysis of glutamine is that they allow of a simple calculation of the free energy of hydrolysis of the terminal pyrophosphate group of adenosine triphosphate.

For the combined determination of the free energy, heat and the entropy changes of an equilibrium reaction with one analytical method of general applicability, Benzinger (1956) suggested making two calorimetric measurements with reactants and products. In the present study, this procedure was extended to study a reaction that goes practically to completion. By measuring the heat produced when glutaminase was added to ammonium glutamate solutions, the reversibility of the glutaminase reaction could be demonstrated. An important advantage of such a calorimetric procedure is that small chemical changes can be followed whatever is the original composition of the reaction mixtures. It was therefore possible to study the formation or disappearance of small amounts of glutamine in mixtures which contained 1000–3000 times as much ammonium glutamate as glutamine.

The free-energy change for the hydrolysis of glutamine has been combined with data for the glutamine synthetase reaction (Levintow & Meister, 1954; Varner & Webster, 1955) to obtain the free energy of hydrolysis of adenosine triphosphate.

The measurements with glutaminase have been briefly reported in an earlier paper (Benzinger & Hems, 1956). These experimental results can now be considered in conjunction with experimental values for the activities of ammonium glutamate in aqueous solution [R. A. Robinson personal communication (1958)] and also with recent values for the formation constants of the magnesium complexes of adenine nucleotides (Burton, 1959).

EXPERIMENTAL

Heat measurements

The microcalorimeter was the same as described in the preceding paper (Kitzinger & Hems, 1959). In each experiment, 16 ml. of the substrate solution (a known mixture of ammonium glutamate, KCl and glutamine) was measured into the main compartment of a microcalorimetric drop vessel (Kitzinger & Benzinger, 1955) and two 0·08 ml. drops of the glutaminase solution (20 mg./ml.) were measured into each of two dimpled recesses of the vessel. The calorimeter was assembled and, when thermal equilibrium was attained, the enzyme and substrate were mixed by rotating the entire calorimeter. The rate of heat flow from the calorimeter vessel and across the thermopile of 10 000 junctions was continuously recorded (Figs. 1, 3 and 4).

In the later experiments of this work, the enzyme was dissolved in ammonium glutamate plus KCl of the same composition as the principal substrate solution. This procedure eliminated most of the mixing heat which occurred with enzyme dissolved in water. Chemical equilibrium was attained in the small volume of enzyme and ammonium glutamate before it was mixed with the bulk of the ammonium glutamate.

The chemical and electrical calibrations carried out between the experiments are described in the preceding paper (Kitzinger & Hems, 1959).

Materials

Glutaminase. This was prepared from *Clostridium welchii* (SR 12) by the method of Hughes & Williamson (1952). To increase the enzyme concentration, the solution obtained after stage 4 (dialysis) was further treated: stage 4 enzyme solution, equivalent to 10 g. of dry bacteria, was freeze-dried and then redissolved in 10 ml. of water. The clear solution was dialysed against 4 l. of 5 mM-KCl for 4 hr. at 2°. The dialysis reduced the small-molecular-weight ions to a minimum, but retained sufficient Cl^- ions to activate the enzyme. The enzyme solution was then freeze-dried, yielding about 28 mg. of dry powder, which was stored at −10°. The preparations had no detectable glutamic acid decarboxylase activity, as shown by the absence of CO_2 production in Warburg manometers or by a negligible heat change in the calorimeter. The enzyme did not liberate detectable quantities of ammonia from a sample of DL-*iso*glutamine.

The dialysis step was not performed for the enzyme used for the experiments of Fig. 1.

L-*Glutamine.* This was the sample used in the preceding paper (Kitzinger & Hems, 1959).

Ammonium glutamate. Solutions were prepared as follows: to 33·1 g. of L-glutamic acid in a 250 ml. volumetric flask was added the calculated quantity of 5N-NH_3 solution to neutralize the acid, and about 180 ml. of water. The flask was shaken until all the solid had dissolved, and 2N-HCl was then added dropwise until the pH, as measured by a glass electrode, had fallen to about 5·4. The volume was made up to 250 ml. with water. This solution contained 0·9M-glutamic acid, 0·9M-NH_3 solution and was 0·0511N with respect to chloride. It was diluted as required with 0·0511M-KCl.

Thermodynamic symbols. These are listed by Burton & Krebs (1953).

RESULTS

Equilibrium of the glutaminase system. In six successive experiments, 0·16 ml. of a glutaminase preparation (concentrated but not dialysed after stage 4) was added to 16 ml. of ammonium glutamate and the heat flow was recorded (Fig. 1). In one run (*f*) no glutamine was added to the glutamate; in all the other experiments (*a*–*e*) small quantities of glutamine had been added to the ammonium glutamate solution before mixing with the enzyme. A substantial absorption of heat occurred when the enzyme was added to pure ammonium glutamate (*f*). Less heat was absorbed in the presence of small amounts of glutamine (*d* and *e*) and heat was liberated with more

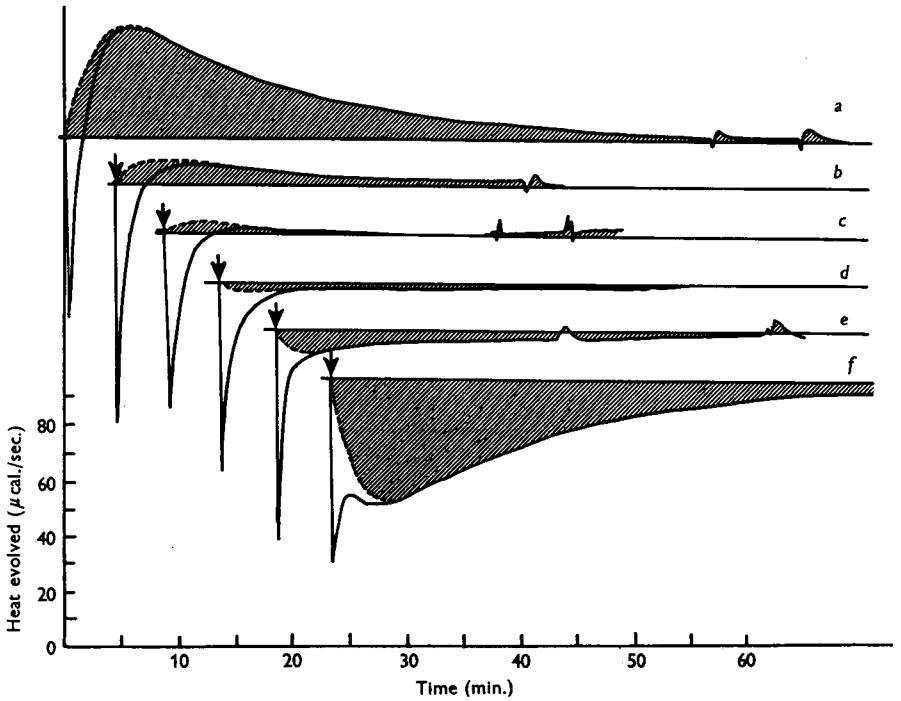

Fig. 1. Tracings of calorimeter recordings. The continuous line is the rate of heat flow recorded after mixing 0·16 ml. of glutaminase with 16 ml. of glutamine solution in 0·884M-ammonium glutamate at 25°. The broken line is the heat flow of the enzymic reaction after correcting for the initial mixing heat; the shaded areas represent the amount of the corresponding heat change. (Above the horizontal line heat was evolved, below the line heat was absorbed.) The initial concentration of glutamine in the 16·16 ml. of solution immediately after mixing was (mM): *a*, 1·57; *b*, 0·923; *c*, 0·876; *d*, 0·785; *e*, 0·672; *f*, zero. Successive tracings have been displaced in the time axis. Arrows indicate time of addition of enzyme, in each experiment.

glutamine (*a–c*). Although the heat flow observed in *d* was almost negligible, the enzyme was subsequently shown to be fully active by the addition of 12·7 μmoles of glutamine.

The recordings show an initial spike of heat absorption (mixing heat) which is due to rapid ionic reactions on mixing the two solutions in the calorimeter. It can be separated from the heat of the enzymic reaction because the mixing spike has the instantaneous rise and exponential decline which are characteristic of recordings obtained on introducing an instantaneous pulse of heat into the instrument (Kitzinger & Benzinger, 1955). As shown by the broken lines and shaded areas, the heat of the enzymic reaction has a slower rise and a slower decline. The discontinuities in the later portions of some of the curves are due to repeated mixing operations, carried out to show the absence of appreciable thermal disturbances associated with the agitation.

In Fig. 2, the rates of heat production or absorption at 12 min. after mixing are plotted against the quantities of glutamine added to the ammonium glutamate solutions. The time of 12 min. was chosen because the mixing heat had then subsided. A linear relationship was found between the rates of reaction and the initial concentrations of glutamine on either side of the equilibrium position. By interpolation (Fig. 2) there would have been no heat change in the presence of 0·83 mM-glutamine. An alternative but less accurate method of calculation is based on the total heat changes. The equilibrium position thus calculated agrees within 1 %.

Two further series were performed, one with 0·444 M- (Fig. 3) and the other with 0·223 M-ammonium glutamate (Fig. 4). For these experiments a dialysis step had been included in the preparation of the enzyme, and, as mentioned above, the enzyme was dissolved in substrate solution instead of in water to reduce further the heats of mixing. The equilibrium quantities of glutamine contained in these enzyme drops have been considered in the evaluation. The amplification of the thermoelectric potential was increased by a factor of two for the experiments of Fig. 4.

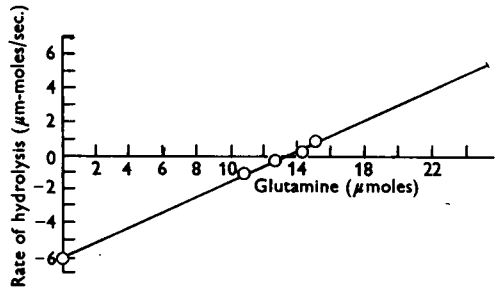

Fig. 2. Graphic evaluation of glutaminase equilibrium. Rates of glutamine hydrolysis and glutamine synthesis are plotted against the initial glutamine in experiments *a* –*f* of Fig. 1. The intercept with no heat change is the amount of glutamine present at equilibrium/16·16 ml.

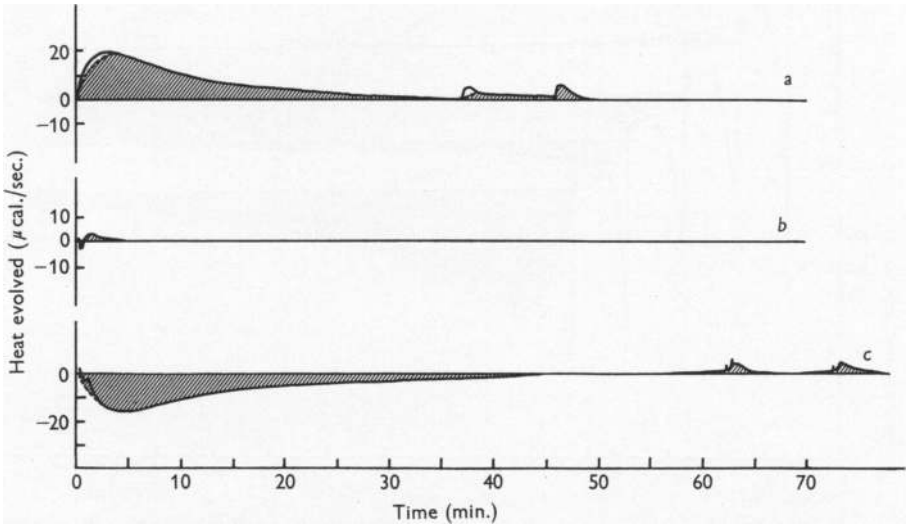

Fig. 3. Tracings of calorimeter recordings. As Fig. 1 but 0·444 M-ammonium glutamate and the enzyme were dissolved in 0·444 M-ammonium glutamate to reduce the heat associated with mixing. Initial concentrations of glutamine added to 16 ml. of ammonium glutamate solution were *a*, 0·530; *b*, 0·241; *c*, zero. Interpolated value for zero heat change is 0·237 mM. No correction is necessary for the 1 % dilution by the enzyme solution since the equilibrium concentration of glutamine should have been formed in the 0·16 ml. of enzyme solution before mixing occurred. The equilibrium concentration is therefore 0·237 mM. Enzyme was added at zero time.

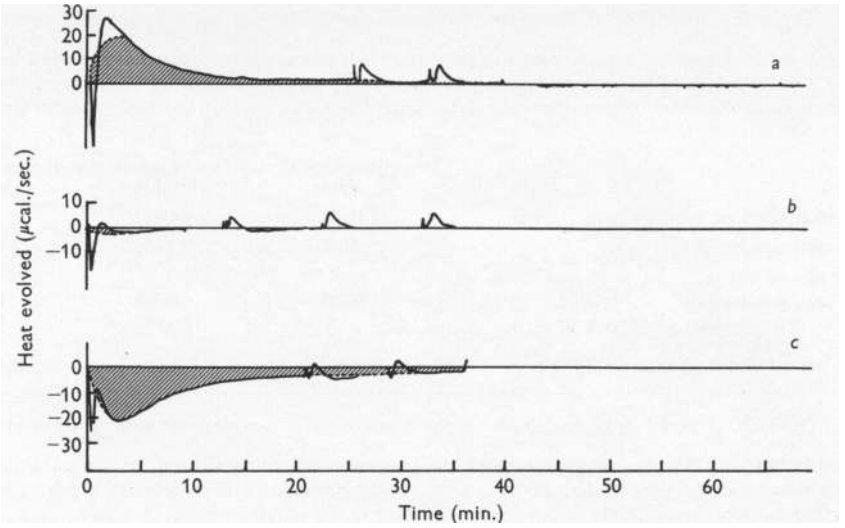

Fig. 4. Tracings of calorimeter recordings. As Fig. 3 but 0·223 M-ammonium glutamate. Initial concentrations of glutamine (mM): *a*, 0·118; *b*, 0·059; *c*, zero. Interpolated concentration for zero heat change 0·066 mM. Enzyme was added at zero time.

Table 1. *Equilibrium of the glutaminase reaction*

Concentrations are those found at equilibrium 25°.

		Figs. 1 and 2	Fig. 3	Fig. 4
Ammonium glutamate	Molarity at 20°	0·884	0·444	0·223
	Density at 20° (g./ml.)	1·0516	1·0302	1·0155
	Molality	0·975	0·464	0·228
	Mean molal activity coefficient	0·543	0·601	0·672
	Activity (*a*) at pH 7·0	0·528	0·283	0·153
	(*b*) at pH 5·5	0·511	0·274	0·148
Activity of water, *a* H_2O (pure water = 1·000)		0·970	0·985	0·993
Glutamine	Millimolarity	0·830	0·237	0·066
	Millimolality	0·917	0·247	0·068
	Molal activity coefficient	0·94	0·96	0·98
	Activity $\times 10^3$	0·86	0·24	0·067
Apparent equilibrium constant (pH 5·5) (moles/1000 g. of water) $K_c = \frac{[\text{Ammonium glutamate}]^2}{[\text{Glutamine}]}$		1040	877	770
Thermodynamic equilibrium constant K_a $K_a = \frac{(\text{Ammonium glutamate})^2}{(\text{Glutamine})\, a_{H_2O}}$		314	318	330

The apparent equilibrium constants were evaluated by graphical interpolation similar to that of Fig. 2 and, as shown in Table 1, there is a marked trend with the different concentrations of ammonium glutamate.

Activity coefficients. Molal activity coefficients for ammonium glutamate have been measured by R. A. Robinson (personal communication, 1958). To use these data, the molar concentrations of ammonium glutamate have been converted into molarities with the aid of measured density values (Table 1). Robinson has measured also the activity of water in solutions of ammonium glutamate. The activity coefficient of glutamine is expected to be close to unity, but, as a check, we have compared the solubilities of glutamine in water and in 0·884 M-ammonium glutamate. The solutions were saturated with glutamine by standing at 20° for 24 hr. with occasional shaking. Portions (1 ml.) of each of these solutions and also of 0·88 M-ammonium glutamate were measured into tared vessels containing known amounts of dry

Table 2. *Solubility of glutamine in water and in* 0·88 M-*ammonium glutamate*

Saturated solutions of glutamine were prepared in (*a*) water and (*b*) ammonium glutamate at pH 7 and 20°. Portions (1 ml.) and also 1 ml. portions of 0·88 M-ammonium glutamate were weighed and dried to constant weight. Weights of ammonium glutamate in the third column were calculated from the water content and the values in the second column.

	Glutamine in water	0·88 M-Ammonium glutamate	Glutamine in 0·88 M-ammonium glutamate
Wt. of 1·00 ml. of solution (g.)	1·0133	1·0521	1·0667
Wt. after drying (g.)	0·0395	0·1437	0·1814
Derived values:			
Wt. of water (g.)	0·9738	0·9084	0·8853
Wt. of ammonium glutamate (g.)	—	0·1437	0·1433
Wt. of glutamine (g.)	0·0395	—	0·0381
Concn. of glutamine (moles/1000 g. of water)	0·278	—	0·295

Table 3. *Thermodynamic data for the hydrolysis of asparagine and glutamine*

From the data of this and the preceding paper with the ionization constants as used by Burton & Krebs (1953). The values appear to be accurate to ±0·1 kcal. or better, except for those marked with an asterisk which have probable errors of 0·2–0·4 kcal. Temp., 25°.

	ΔG^0 (kcal.)	ΔH^0 (kcal.)	$T\Delta S^0$ (kcal.)
Glutamine + H_2O → glutamate^{+-} + NH_4^+	−3·42	−5·16	−1·73
Asparagine + H_2O → aspartate^{+-} + NH_4^+	−3·6*	−5·71	−2·1*
Glutamine + H^+ + H_2O → glutamic acid + NH_4^+	−9·32	−6·11	3·21*
Asparagine + H^+ + H_2O → aspartic acid + NH_4^+	−8·9*	−7·24	1·7*

Celite (about 0·5 g.). The vessels plus contents were then weighed and dried to constant weight over P_2O_5 in a desiccator evacuated to 0·1 mm. Hg. The Celite increased the rate of drying, presumably by reducing the occlusion of water by the crystals of ammonium glutamate. The compositions of the saturated solutions were calculated from the losses in weights as shown in Table 2. The molal concentration of glutamine was about 6 % greater in the ammonium glutamate solution and thus the activity coefficient of glutamine is 0·94 times that in water. For the present purposes this value should not be substantially different at the much lower concentrations of glutamine which are present in the equilibrium mixtures. Interpolated values have been taken in Table 1 for the lower concentrations of ammonium glutamate.

It will be seen that, despite the large variation between the apparent equilibrium constants, the thermodynamic constants agree very well after correction for the activity coefficients:

$$K_a = 320 \pm 3\,\%.$$

DISCUSSION

The reliability of the values for the equilibrium constants depends on the absence of side reactions which evolve or absorb heat. The more obvious possibilities are eliminated by the absence of any action on sodium glutamate or *iso*glutamine, and by the agreement between the thermodynamic-equilibrium constants at the different concentrations of ammonium glutamate. The shapes of the heat flow–time curves are also evidence against the occurrence of side reactions. Thus those not involving glutamine would have reversed the sign of the heat production during experiment *c* or *d* of Fig. 1, except in the improbable case that the time course of the side reaction was similar to that of the glutaminase reaction. A side reaction involving glutamine would have produced different shapes of the heat flow–time curves depending on the direction from which equilibrium was approached. Such a difference is not seen in the comparison of curves *a* and *f* or *b* and *e* of Fig. 1.

The equilibrium constant obtained ($K_a = 320$ at 25°) corresponds to a free-energy change of −3·42 kcal. for the reaction:

$$\text{Glutamine} + H_2O \rightarrow \text{glutamate}^{+-} + NH_4^+. \quad (1)$$

We consider ±10 % in the thermodynamic-equilibrium constant or ±0·06 kcal. in the free-energy change a reasonable estimate of the total possible error, taking into account probable errors in preparing and measuring solutions and in the activity coefficients of ammonium glutamate and glutamine.

From the heat of reaction (Kitzinger & Hems, 1959), ΔG^0 is calculated to be −3·35 kcal. at 37° and the equilibrium constant is 225.

Comparison between asparagine and glutamine. The thermodynamic data for the hydrolysis of the two amides are shown in Table 3. Although the ΔG^0 values are fairly close, the ΔH^0 and ΔS^0 values

differ substantially from each other when the products are the acids, but less when they are the aspartate^{+--} and glutamate^{+--} ions. The greatest differences are under the conditions where the closest agreement might have been expected (Morales, Botts, Blum & Hill, 1955).

Free energy of hydrolysis of adenosine triphosphate. To derive this we use the data of Levintow & Meister (1954) and of Varner & Webster (1955) for the equilibrium of the glutamine synthetase reaction. The equilibrium is complicated by the formation of complexes with Mg^{2+} ion which is an essential component of the glutamine synthetase system. In most of Levintow & Meister's measurements, the equilibrium mixtures contained approximately 9 mM-adenosine diphosphate (ADP), mM-adenosine triphosphate (ATP), 9 mM-inorganic orthophosphate, 9 mM-glutamine, mM-ammonium glutamate, 50 mM-$MgCl_2$, about 30 mM-Na^+ ion, 2-amino-2-hydroxymethylpropane-1:3-diol (tris) or imidazole buffer and the enzyme.

Only the phosphate compounds bind appreciable amounts of Mg^{2+} ion, since (1) the pH of either of the buffers is not appreciably altered by 0·2 M-$MgCl_2$ and (2) less than 0·03 atom of Mg is bound/mole of ammonium glutamate at pH 7 and 12·5 mM-free Mg^{2+} ion, as shown by spectrophotometric measurements made with 8-hydroxyquinoline according to the procedure of Burton (1959). Glutamine is expected to bind less strongly than the glutamate ion by analogy with the behaviour of asparagine and the aspartate ion (Albert, 1952).

On the other hand, virtually all of the ATP and ADP would be present as magnesium complexes (see Burton, 1959). Assuming that the formation of bimetallic complexes such as Mg_2ATP is not appreciable, 10 mM-Mg^{2+} ion will be bound by the nucleotides. Smith & Alberty (1956*a*, *b*) have studied the binding of cations by inorganic orthophosphate at 25° and their results indicate that the total of 9 mM-phosphate would be divided into 4·8 mM-$MgHPO_4$, 0·22 mM-$NaHPO_4^-$, 1·81 mM-$H_2PO_4^-$ and 2·18 mM-HPO_4^{2-}. The measurements of Clarke, Cusworth & Datta (1954) show that these values would be slightly altered at 37° so that the concentrations would be about 5·2 mM-$MgHPO_4$ and 1·58 mM-HPO_4^{-2}. Thus there would remain about 35 mM-free Mg^{2+} ion in the glutamine synthetase-equilibrium mixtures.

In a personal communication, Levintow has recently confirmed that the best value from Levintow & Meister's measurements of the equilibrium constant was

$$K = \frac{[\text{ADP}]\,[\text{inorganic phosphate}]\,[\text{glutamine}]}{[\text{ATP}]\,[\text{NH}_4]\,[\text{glutamate}]} = 1200 \text{ at pH } 7{\cdot}0 \text{ and } 37°.$$

The few measurements at 22° instead of 37° or at different pH values are considered less reliable because the reduced activity of the enzyme under these conditions made it uncertain that true equilibrium conditions were reached. The concentrations used to obtain this equilibrium constant are the total concentrations, including all the ionic species and complexed forms of each compound. The total ionic strength in the equilibrium mixtures is calculated to be about 0·2, and the mean ion activity coefficient of ammonium glutamate is therefore 0·69 [Robinson, personal communication]. The activity coefficient of the glutamine is taken to be unity. We can therefore convert the above equilibrium constant into the following one:

$$K' = \frac{[\text{MgADP}^-]\,[\text{HPO}_4^{2-}]\,(\text{glutamine})}{[\text{MgATP}^{2-}]\,(\text{NH}_4^+)\,(\text{glutamate}^{+--})} = \frac{1200 \times 1{\cdot}58}{(0{\cdot}69)^2 \times 9{\cdot}0} = 442 \text{ at pH } 7{\cdot}0 \text{ and } 37°.$$

As is customary, brackets indicate concentrations and parentheses indicate thermodynamic activities.

When equilibrium was approached from the other side, Levintow & Meister found a lower value for their observed equilibrium constant (730 instead of 1200; $K' = 270$), and it seemed that equilibrium had not been attained because the reaction was very slow. Varner & Webster (1955) have also studied the equilibrium from this side. Their observed equilibrium constant is 1700 at pH 7·4 and 35° and an ionic strength of about 0·15. When converted in the same way as above, we find $K' = 900$ at pH 7·4 and 35° (the values used in this conversion are: free Mg^{2+} ion = 25 mM; $MgHPO_4 = 3{\cdot}1$ mM; $HPO_4^{2-} = 1{\cdot}37$ mM; total inorganic phosphate = 5 mM; $\gamma\pm = 0{\cdot}72$ for ammonium glutamate). The constant K' becomes 360 at pH 7·0 because a H^+ ion is involved in the net reaction:

$$\text{MgATP}^{2-} + \text{NH}_4^+ + \text{glutamate}^{+--} = \text{glutamine} + \text{MgADP}^{2-} + \text{HPO}_4^{2-} + \text{H}^+. \quad (2)$$

On the basis of these values we take $K' = 400 \pm 120$ at pH 7·0 and 37°. The allowance for error is an estimate of the total error and it includes allowances for the deviations reported by Levintow & Meister as well as uncertainty about the attainment of equilibrium. When this value is combined with that of 225 ± 22 for the thermodynamic equilibrium constant of the glutaminase reaction at 37° we can calculate the equilibrium constant at pH 7·0, 37° and ionic strength about 0·2;

$$K = \frac{[\text{MgADP}^-]\,[\text{HPO}_4^{2-}]}{[\text{MgATP}^{2-}]} = 9 \pm 4 \times 10^4 \text{ M}.$$

This corresponds to $\Delta G' = -7{\cdot}0 \pm 0{\cdot}17$ kcal. for the reaction

$$\text{MgATP}^{2-} + \text{H}_2\text{O} \rightarrow \text{MgADP}^{2-} + \text{HPO}_4^{2-} + \text{H}^+. \quad (3)$$

This is reasonably close to the estimate of $-7{\cdot}46$ kcal. (30°) derived from the hexokinase (Robbins & Boyer, 1957) and glucose 6-phosphatase equilibria (Meyerhof & Green, 1949; see Robbins & Boyer, 1957). Appreciable errors may, however, have been introduced into this estimate by assumptions about the activity coefficients for the equilibrium mixtures of the glucose 6-phosphatase reaction.

With no magnesium, reaction (3) becomes

$$ATP^{4-} + H_2O \rightarrow ADP^{3-} + HPO_4^{2-} + H^+ \quad (4)$$

and, to obtain its $\Delta G'$, the formation constants are needed for the magnesium complexes of ATP and ADP. Previously (Burton & Krebs, 1953; Robbins & Boyer, 1957; Burton, 1958) the values used for these constants indicated that $\Delta G'$ of reaction (4) was more negative than that for reaction (3) by 0·5 kcal. (Burton & Krebs, 1953) or 0·7 kcal. (Smith & Alberty, 1956*b*). The difference is similar to Nanninga's (1957) values for the formation constants but considerably larger if the values of Martell & Schwarzenbach (1956) or of Walaas (1957) are used. The larger difference has recently been confirmed by Burton (1959), whose results indicate a difference of 1·7 kcal. at 37° and ionic strength 0·1. The difference appears to be smaller at higher ionic strengths so, taking 1·6 kcal. for an ionic strength of 0·2, the value of $-8{\cdot}6$ kcal. is obtained for $\Delta G'$ of reaction (4) at pH 7 and 37° ($-9{\cdot}3$ kcal. at pH 7·5). With the aid of the known heat of reaction (Kitzinger & Benzinger, 1955; Podolsky & Morales, 1956) these values can be converted into other temperatures (e.g. $-9{\cdot}1$ kcal. at pH 7·5 and 25°).

The value at pH 7·5 is given because of its convenience for free-energy calculations. It so happens that, at this pH, there is little difference between the value of $\Delta G'$ (which applies to M-concentrations of the specific ionic forms) and the value which applies to M-total concentration of each phosphate compound, whereas there is a larger difference at pH 7.

As pointed out in a recent brief survey (Burton, 1958), the more reliable estimates for the free energy of hydrolysis of ATP are in reasonable agreement with each other. In most of these estimates, the effects of the binding by Mg^{2+} ions was ignored and when these effects were considered, the data of Burton & Krebs (1953) or of Smith & Alberty (1956*b*) were used. The larger effect of the magnesium-binding means that the estimates quoted by Burton (1958) for reaction (4) should all be changed in the same direction by an amount between 0·7 and 1·6 kcal. This correction does not destroy the general agreement between the more reliable of the estimates. The estimates quoted for the free-energy changes in living cells or during oxidative phosphorylation will not be altered if, as seems probable, ATP and ADP are mostly present as their metal complexes.

SUMMARY

1. Reversal of the enzyme-catalysed hydrolysis of the amide group of glutamine has been studied by following the heat changes by a sensitive calorimeter. At pH 5·5 and 25°, the apparent equilibrium constants were found to be 1040, 885 or 765 moles/1000 g. of water with 0·885, 0·444 or 0·223 M-ammonium glutamate respectively.

2. This trend disappeared after correcting for the activity coefficients. The respective thermodynamic equilibrium constants become 314, 318 and 330 at pH 7 and 25° and the free energy of hydrolysis is therefore $-3{\cdot}42 \pm 0{\cdot}06$ kcal./mole.

3. By the use of equilibrium data for the glutamine synthetase reaction (Levintow & Meister, 1954; Varner & Webster, 1955), the free energy of hydrolysis of $MgATP^{2-}$ to $MgADP^-$ and HPO_4^{2-} ions is found to be $-7{\cdot}0$ kcal. at pH 7 and 37°.

4. In the absence of magnesium, the value of $-9{\cdot}3$ kcal. (pH 7·5, 37°) has been obtained for the free energy of hydrolysis of ATP^{4-} to ADP^{3-} and HPO_4^{2-} ions.

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Studies on Carbohydrate-Metabolizing Enzymes

TRANS-β-GLUCOSYLATION BY BARLEY ENZYMES

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Recent investigations (reviewed by Gottschalk, 1958) have shown that the enzymic hydrolysis of a glycosidic linkage in an oligosaccharide is, in fact, a transglycosylation reaction in which a glycosyl group is transferred from the substrate to a water molecule. The latter thus acts as a glycosyl acceptor:

$$G\text{–}O\text{–}X + \text{Enzyme–H} \rightarrow \text{Enzyme–G} + H\text{–}O\text{–}X, \quad (1)$$

$$\text{Enzyme–G} + H\text{–}O\text{–}R \rightarrow \text{Enzyme–H} + G\text{–}O\text{–}R, \quad (2)$$

where R is a hydrogen atom, G–O–X represents the substrate (glycosyl donor) and H–O–R the glycosyl acceptor. Other compounds containing hydroxyl groups (e.g. carbohydrates or alcohols) may function as glycosyl acceptors, and with these the synthesis of a new saccharide or glycoside is observed. In the transglycosylation reaction the configuration of the glycosidic linkage is retained. The hydrolytic and 'transferase' activity of a carbohydrase thus represent two examples of the same enzymic reaction, and the enzyme will show a dual specificity towards the glycosyl donor and the acceptor.

During a study of barley β-glucosidases, Manners (1955*a*) found that whereas cellobiose in dilute aqueous solution was completely hydrolysed to glucose, in concentrated solution the production of glucose was accompanied by oligosaccharide synthesis. The latter could arise by the enzymic transfer of β-glucosyl radicals to glucose and cellobiose. This paper describes the characterization of these oligosaccharides, and this has enabled the acceptor specificity of barley cellobiase to be determined. For convenience, the enzyme system catalysing reactions (1) and (2) with cellobiose as β-glucosyl donor will be referred to as cellobiase, although purification has not been attempted and information on the number of active factors is not available.

METHODS AND MATERIALS

Analytical methods

Chromatography. (*a*) Qualitative. Descending paper chromatograms were prepared at room temperature with Whatman no. 1 paper and either ethyl acetate–pyridine–water (10:4:3, by vol.) (*A*) or propanol–ethyl acetate–water (6:1:3, by vol.) (*B*) as solvent. Aniline oxalate or silver nitrate–sodium hydroxide was used as spray reagent. The rate of movement of sugars is expressed relative to D-glucose (R_G values).

(*b*) Preparative. Charcoal–Celite columns were prepared as previously described (Duncan & Manners, 1958).

Electrophoresis. Separation of oligosaccharides on Whatman no. 1 paper was effected in an apparatus similar to that described by Foster (1952) at 750 V and 10 mA. 0·1 N-Borate buffer (pH 10·0) was used.

Acid hydrolysis. Complete acid hydrolysis of oligosaccharides was carried out by heating with 1·5 N-sulphuric acid (0·5% solution) at 100° for 3 hr. Reducing sugars were determined with the Somogyi (1952) reagent calibrated against glucose and cellobiose. Partial acid hydrolysis of oligosaccharides and the derived aldonic acids was effected by the methods of Duncan & Manners (1958).

Periodate oxidation. The consumption of periodate, production of formic acid, and formaldehyde were determined by the methods of Fleury & Lange (1933), Halsall, Hirst & Jones (1947) and Hough, Powell & Woods (1956), respectively.

Acetylation of sugars. The oligosaccharide (about 50 mg.) was heated at 135° with sodium acetate (80 mg.) and acetic anhydride (1·5 ml.) until solution was effected, and then for a further 15 min. The acetylated product was separated by pouring into ice-cold water (5–10 ml.), purified by solution in chloroform and recrystallized from methanol.