induced by dCTP and by glycerol while the conformation of the inhibited enzyme can be induced by dTTP and by high ionic strength. The demonstration reported in the preceding paper (Geraci *et al.*, 1967) that no polymer–monomer conversion occurs in the presence of dCTP and of dTTP, strengthens the hypothesis that the changes of enzyme activity caused by the regulatory effectors, are based only on conformational changes of the enzyme molecule.

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Effect of Salt Solutions on Glycogen Phosphorylase. A Possible Role of the Phosphoryl Group in Phosphorylase a*

Robert W. Sealock† and Donald J. Graves‡

ABSTRACT: The structure and catalytic activity of phosphorylases a and b have been found to be particularly sensitive to salts. Kinetic studies with respect to adenosine monophosphate (AMP) for phosphorylase b indicate that the saturation curve is hyperbolic in NaF but sigmoidal in its absence with a Hill coefficient of $n = 1.5$. In contrast to NaF, phosphorylase b is less active in $NaClO_4$ and does not follow Michaelis–Menten kinetics for AMP. These salts had little effect on the sedimentation of phosphorylase b in the absence of AMP, but in the presence of 10^{-3} M AMP and NaF, the enzyme sedimented as a tetramer, and in AMP and $NaClO_4$, as a mixture of dimer and tetramer In NaF phosphorylase a is stimulated little by AMP, but in $NaClO_4$ the requirement for AMP for activity is nearly complete. NaF has no effect on the sedimentation of phosphorylase a, but in $NaClO_4$ the enzyme is partially dissociated to a dimeric form. A model, based upon the assumed effect of the covalently bound phosphate groups of phosphorylase a on activity coefficients of certain groups at a specific site, is proposed to explain the differences in structure and activity of phosphorylases a and b. The action of salts is discussed in relation to this model.

It is well recognized that the interconversion of phosphorylases b and a from skeletal muscle arises from specific enzymic phosphorylation and dephosphorylation reactions and that these transformations constitute, in part, an important mechanism by which glycogen metabolism is controlled *in vivo*. The incorporation of phosphate that occurs in the phosphorylase b to a reaction (Krebs and Fischer, 1956) results in an enzyme that differs from its precursor in sensi-

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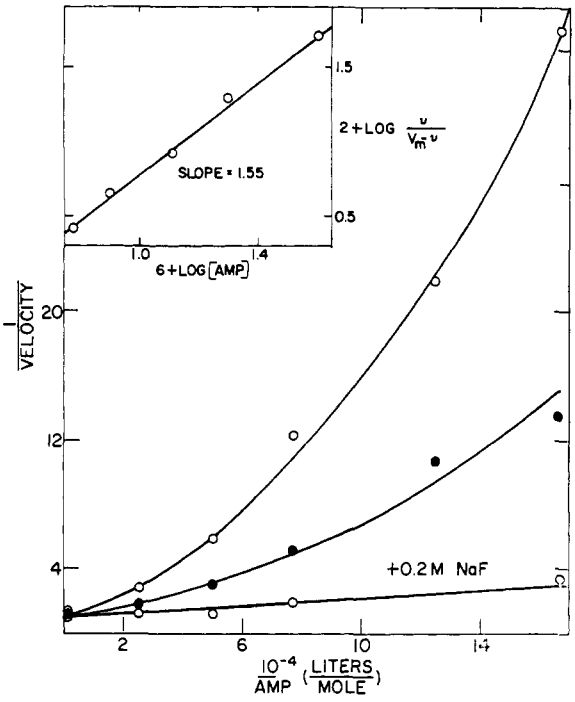

FIGURE 1: Reciprocal plot for AMP in the phosphorylase b reaction. Reaction mixtures contained 0.015 M cysteine–0.02 M glycerophosphate buffer at pH 6.7, enzyme (37 μg/ml), 1% glycogen, AMP, 0.015 M glucose-1-P (○), or 0.050 M glucose-1-P (●), and 0.2 M NaF as indicated.

tivity to AMP[1] (Green and Cori, 1943), kinetic constants (Helmreich and Cori, 1964; Lowry *et al.*, 1964), quaternary structure (Keller and Cori, 1953), and thermal stability (Graves *et al.*, 1965). Although much is known about the chemistry and physiological significance of these transformations, it is unknown on a molecular basis, why the nonphosphorylated form of the enzyme, phosphorylase b, is catalytically active only in the presence of AMP, or why phosphorylation of two seryl residues per molecule of phosphorylase b results in a molecule which is active in the absence of AMP. In the present work, salt solutions have been used as a tool to examine the effect of various ionic environments on certain catalytic and physical properties of phosphorylases a and b from rabbit muscle in order to provide some clue as to the nature of the role of phosphate groups in phosphorylase a.

Experimental Procedures

Materials. Phosphorylase b was isolated according to the procedure of Fischer and Krebs (1958). Phosphorylase a was prepared from crystalline phosphorylase b and phosphorylase kinase, according to Fischer and Krebs (1962). The specific activities of enzyme used in this work ranged from 1300 to 1800 Cori units/mg for phosphorylase b and from 1800 to 2000 Cori units/mg for phosphorylase a as measured by the procedure of Illingworth and Cori (1953). Reduced phosphorylase b was prepared as described previously (Graves *et al.*, 1965).

AMP was purchased from Pabst Laboratories. All salts were reagent grade and were used without further purification. Cysteine·HCl, sodium glycerophosphate, potassium glucose 1-phosphate, and shell fish glycogen were products of Sigma Chemical Co. The glycogen and enzyme solutions were treated with Norit A to remove contaminating AMP.

Methods. For determination of initial velocities, enzyme solutions were preincubated at 30° for 1–2 hr in 0.04 M glycerophosphate–0.03 M cysteine, pH 6.7–6.8, prior to rate measurements. Reactions with phosphorylases b and a were run at 30° with substrate containing 1% glycogen, glucose-1-P, AMP, and salt as indicated. Aliquots were removed and analyzed for inorganic phosphate according to Fiske and Subbarow (1925). Sampling times were adjusted to insure linear product *vs.* time curves. All velocities are expressed as micromoles of inorganic phosphate released per minute per milliliter. Protein concentration was determined spectrophotometrically with the use of an absorbance index of 11.7 for a 1% solution of protein (Velick and Wicks, 1951).

Ultracentrifuge runs were performed on a Spinco Model E at a rotor speed of 59,780 rpm and a temperature of 20 ± 1°. Sedimentation coefficients were determined with the aid of a Nikon Model 6C microcomparator and were corrected for viscosity and density of the buffer to water at 20°.

Results

Effect of Salts on the Catalytic Properties of Phosphorylase b. Robinson and Jencks (1965) showed that a correlation exists between the effect of salt solutions on protein structure and on the activity coefficient (solubility) of the peptide, acetyltetraglycine ethyl ester (ATGEE).[1] A similar correlation has now been found to apply to effects of a number of salts on phosphorylase activity. Therefore, the alterations of catalytic and physical properties of phosphorylase by NaF and $NaClO_4$, two salts having opposing effects on the solubility of ATGEE, were examined.

In Figure 1 Lineweaver–Burk plots are shown for AMP in the reaction catalyzed by phosphorylase b which clearly indicate that the saturation curve for AMP and phosphorylase b is sigmoidal at 0.015 and 0.05 M glucose-1-P over the range of AMP concentrations examined (0.6–50 × 10^{-5} M). A similar result was obtained by Helmreich and Cori (1964) in the direction of glycogen breakdown. The insert of Figure 1 shows a plot according to the modified Hill equation of Atkinson *et al.* (1965). The slopes indicate a Hill coefficient of $n = 1.55$. This parameter (and therefore the sigmoidicity of the saturation curve) does not

[1] Abbreviations used: ATGEE, acetyltetraglycine ethyl ester; AMP, adenosine monophosphate.

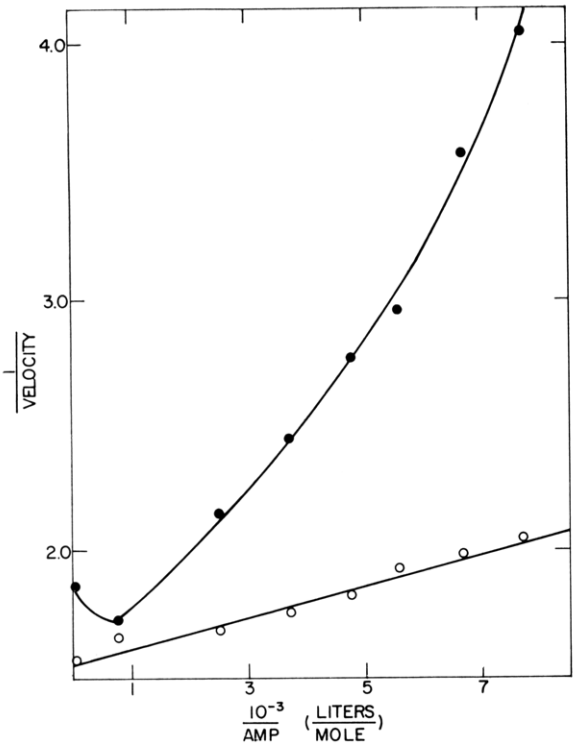

FIGURE 2: Reciprocal plot for AMP in the phosphorylase b reaction. Reaction mixtures were as in Figure 1, but with 15 μg of enzyme/ml. No added salt (○); with 0.05 M $NaClO_4$ (●).

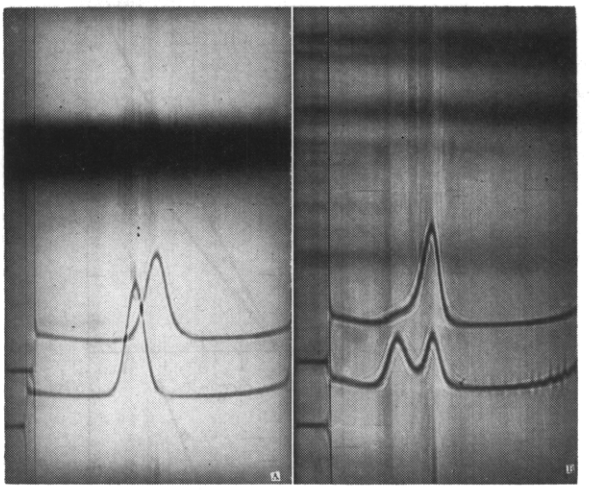

FIGURE 3: Ultracentrifugation of phosphorylase b. Enzyme (10 mg/ml) was centrifuged at 59,780 rpm at 20° in 0.04 M glycerophosphate–0.03 M cysteine buffer at pH 6.7. Pictures were taken approximately 1 hr after preparation of samples. Direction of sedimentation is to the right. (A) Upper curve, 0.1 M $NaClO_4$; lower curve, 0.2 M NaF. (B) Upper curve, 0.1 M NaF and 10^{-3} M AMP; lower curve, 0.2 M $NaClO_4$ and 10^{-3} M AMP.

appear to be a function of glucose-1-P concentration; the average value of n for seven determinations over a range of glucose-1-P concentrations from 9.6 to 64 mM was 1.5, with a standard deviation of 0.13. Also indicated in Figure 1 is the marked activation of phosphorylase b by 0.2 M NaF which is most apparent at low concentrations of AMP. The reciprocal plots with respect to AMP in the presence of NaF are linear and give an apparent K_M value for AMP of $2.0 \pm 0.7 \times 10^{-5}$ M (based on six determinations). Although NaF activates phosphorylase b, it does not appear to substitute for AMP. In 0.35 M NaF without added AMP, phosphorylase b possessed only 2.5% of its catalytic activity as measured in the presence of saturating AMP without fluoride. This small amount of activity is probably owing to residual contamination of the glycogen and enzyme solutions by AMP or the enzyme solutions by phosphorylase a.

In contrast to the above results, those illustrated in Figure 2 show that the reciprocal plot for AMP in 0.05 M $NaClO_4$ is definitely nonlinear at much higher AMP concentrations (*cf.* Figure 1), at which the reciprocal plot in the absence of $NaClO_4$ is best approximated by a straight line. At higher concentrations of AMP (2.5×10^{-2} M) there is a slight but reproducible inhibition of enzyme activity in 0.05 M $NaClO_4$ which is not observed in buffer alone or buffer plus NaF. It should be pointed out that the effects of $NaClO_4$ are more pronounced at higher salt concentrations, but denaturation during the assay, as found through the method of Selwyn (1965), prevented accurate determination of initial velocities.

Effects of NaF and $NaClO_4$ on the Structure of Phosphorylase b. The effect of salts on the ultracentrifugal properties of phosphorylase was investigated. The native enzyme used in these experiments sedimented in glycerophosphate–cysteine buffer, pH 6.7, as a single sharp peak ($s_{20,w}$ = 8.6 S); in buffer with 10^{-3} M AMP the peak was considerably broadened, with an $s_{20,w}$ of 10.1 S, as previously reported by Appleman (1962). In the absence of AMP, the addition of 0.2 M NaF (Figure 3a, lower curve) had no apparent effect on the sedimentation pattern ($s_{20,w}$ = 8.6 S), while addition of 0.1 M $NaClO_4$ (Figure 3a, upper curve) caused the peak ($s_{20,w}$ = 9.1 S) to broaden slightly. The different states of the protein in these salts are most apparent when ultracentrifugation is carried out in the presence of 10^{-3} M AMP (Figure 3b). In 0.1 or 0.2 M NaF and 10^{-3} M AMP (upper curve) the enzyme is present almost exclusively as a more rapidly sedimenting species ($s_{20,w}$ = 12.9 S). By contrast, in 0.2 M $NaClO_4$ and AMP (lower curve) the protein is divided approximately equally between the heavier and lighter species ($s_{20,w}$ = 9.2 and 13.7 S). These two species are presumed to be the normal phosphorylase b dimer and a tetrameric form of the enzyme, similar to the Mg^{2+} AMP generated tetrameric form observed at low temperature (Kent *et al.*, 1958).

Although salts and AMP markedly affect ultracentrifugal and catalytic properties of phosphorylase, it is of interest to note that the optical activity of enzyme-

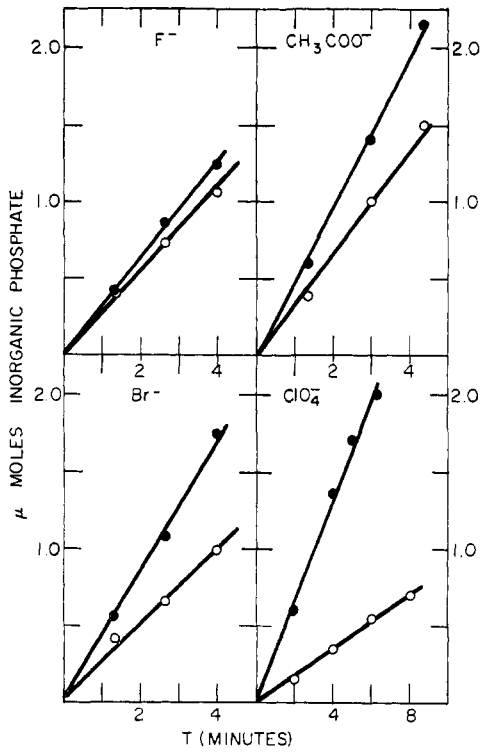

FIGURE 4: Effect of anions on phosphorylase a activity. Reaction mixtures contained enzyme (50 μg/ml) in 0.025 M imidazole–chloride at pH 6.8, 0.01 M glucose-1-P, 1% glycogen, and 0.1 M sodium salts of either F^-, CH_3COO^-, Br^-, or ClO_4^-, and 0.001 M AMP (●). Substrate without AMP (○). Aliquots (0.4 ml) were removed at various intervals and analyzed for inorganic phosphate.

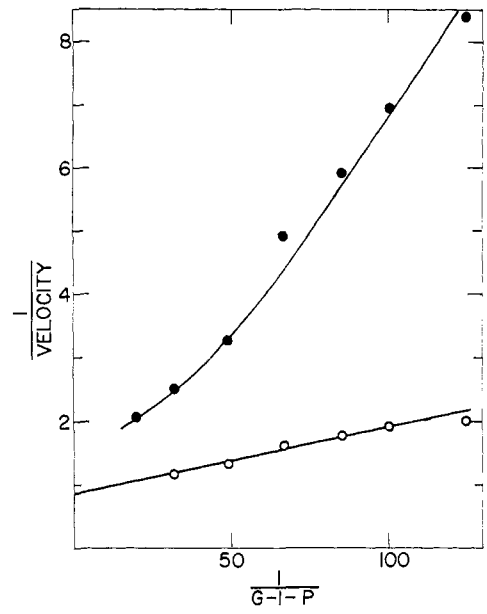

FIGURE 5: Reciprocal plot for glucose-1-P in the phosphorylase a reaction. Reaction mixtures contained enzyme (17 μg/ml) in 0.02 M glycerophosphate–0.015 M cysteine at pH 6.8, 1% glycogen, 0.1 M $NaClO_4$, glucose-1-P as indicated, and 10^{-3} M AMP (○); substrate without AMP (●).

bound pyridoxal phosphate, as indicated by circular dichroism measurements, is identical in the presence of 10^{-3} M AMP, or 10^{-3} M AMP and 0.1 M F^-, or 10^{-3} M AMP and 0.2 M ClO_4^-. In addition, $NaBH_4$-reduced enzyme, in which the prosthetic group is fixed to the protein as a pyridoxamine derivative with concomitant 60% loss of circular dichroism (Johnson and Graves, 1966), shows kinetics for AMP (n = 1.68) similar to those for native enzyme (in keeping with the findings of Fischer *et al.*, 1963) and is also activated by fluoride. These data suggest that the observed changes in catalytic activity and over-all structure of the enzyme in salts occur independently of the local protein environment of the prosthetic group.

Effect of Salts on the Catalytic Properties of Phosphorylase a. Because it is known that the presence of covalently bound phosphate is not essential for phosphorylase activity and that the phosphoryl groups of phosphorylase a do not exchange with inorganic phosphate during enzymic catalysis (Krebs *et al.*, 1958), it has been generally assumed that the phosphoryl groups are not at the active site but are important for stabilization of an active conformation. In order to determine if the effect of the phosphoryl groups are at all related to salting-out properties of inorganic phosphate on proteins and ATGEE, the activity of phosphorylase a has been examined in salt solutions with different solubilizing characteristics. Figure 4 shows that both enzyme activity and the degree of stimulation by AMP depend markedly on anions included in the reaction mixture. With 0.1 M F^-, CH_3COO^-, Br^-, and ClO_4^- activities with AMP were 0.79, 1.20, 1.05, and 0.85 μmoles of phosphate/ml per min, respectively; without AMP activities were 0.68, 0.93, 0.61, and 0.22, respectively. The illustrated linear progress curves show that changes in enzyme activity are not due to differences in enzyme stability in the assay. Interestingly, phosphorylase a in F^- and CH_3COO^-, salts which, like phosphate, salt out ATGEE (Robinson and Jencks, 1965), was stimulated little by AMP; phosphorylase a in Br^-, a salt with no effect on ATGEE solubility, and in ClO_4^-, a salt which salts in ATGEE, showed increasing stimulation of activity by AMP, respectively.

Further study of the effect of perchlorate on phosphorylase a activity in the presence and absence of AMP is indicated in Table I. Although enzymic activity (+AMP) depends upon perchlorate concentration and is reduced approximately 4.5-fold by increase of perchlorate from 0.02 to 0.2 M, these data show that activity (−AMP) is far more sensitive to per-

TABLE 1: Phosphorylase a Activity in $NaClO_4$.

Reaction Mixture[a]	Specific Activity at 20°		Ratio of Act. (−AMP/ +AMP)	% Act. (−AMP)
	−AMP	+AMP		
In 0.02 M $NaClO_4$	9.0	14.5	0.62	100[b]
In 0.2 M $NaClO_4$	0.13	3.3	0.04	1.4
Enzyme diluted from 0.2 to 0.02 M $NaClO_4$	7.3	11.2	0.65	81

[a] Reaction mixtures at pH 6.8 contained glucose-1-P (0.016 M), glycogen (1%), glycerophosphate (0.020 M), cysteine (0.015 M), AMP (0.001 M), and $NaClO_4$ as indicated. Enzyme in 0.02 M $NaClO_4$ (0.048 mg/ml); enzyme in 0.2 M $NaClO_4$ (0.48 mg/ml); diluted enzyme (0.048 mg/ml). Specific activity is expressed as micromoles of inorganic phosphate released per minute per milligram of protein. [b] Per cent activity in 0.02 M $NaClO_4$ was arbitrarily assigned as 100.

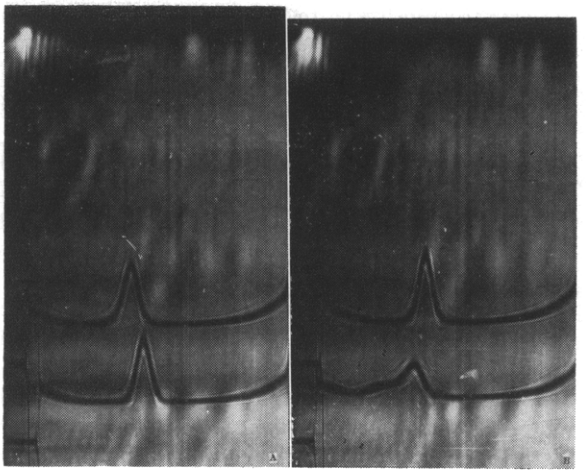

FIGURE 6: Ultracentrifugation of phosphorylase a. Experimental conditions were as in Figure 4. The protein concentration was 5 mg/ml. (A) With 0.2 M NaF. (B) With 0.2 M $NaClO_4$. Lower curves, no AMP; upper curves, with 10^{-3} M AMP.

chlorate. It should be noted that the nearly complete requirement of phosphorylase a for AMP for activity in 0.2 M $NaClO_4$, as indicated by ratio of activities, (−AMP/+AMP), is characteristic of native phosphorylase b. This change in AMP requirement is not a result of irreversible denaturation or a dephosphorylation as observed in the conversion of phosphorylase a to b by phosphorylase phosphatase, since dilution of enzyme after 20 min in 0.2 M $NaClO_4$ into 0.02 M $NaClO_4$ resulted, in this case, in 81% return of activity (−AMP) and a complete reversal of the ratio of activities.

Since fluoride and perchlorate have different effects on phosphorylase a, it was of interest to determine how these salts affected kinetic constants for nucleotide and substrate. With 0.1 M salts and varying AMP from 2.5×10^{-4} to 2×10^{-6} M for fluoride and from 2.5×10^{-4} to 1.5×10^{-7} M for perchlorate, apparent K_M values for AMP were 1.2 and $1.4 \pm 0.4 \times 10^{-6}$ M, respectively. No deviation from linearity was observed in double-reciprocal plots with AMP except with 0.1 M perchlorate at rather low AMP concentrations (below 1.0×10^{-6} M). Further kinetic studies, however, were not pursued since the experimental error in determining initial velocities was too great under these conditions. A Lineweaver–Burke plot for glucose-1-P in the presence of 0.1 M perchlorate is illustrated in Figure 5. In the presence of AMP double-reciprocal plots are linear, giving an apparent K_M for glucose-1-P of 1.0×10^{-2} M. No K_M for glucose-1-P in the absence of AMP was evaluated since double-reciprocal plots were slightly nonlinear. Although it cannot be ascertained from these data whether the V_M with and without AMP in 0.1 M perchlorate are identical, the degree of stimulation of phosphorylase a by AMP depends markedly on glucose-1-P concentration as indicated earlier by Lowry *et al.* (1964). With 0.1 M fluoride, linear double-reciprocal plots were obtained in the absence of AMP also giving an apparent K_M value for glucose-1-P of 1.0×10^{-2} M.

Effect of NaF and $NaClO_4$ on the Ultracentrifugal Properties of Phosphorylase a. Because of the different native quaternary structures and responses to salts of phosphorylases a and b, the effect of NaF and $NaClO_4$ on the ultracentrifugal patterns of phosphorylase a was also investigated. Phosphorylase a in 0.2 M NaF, in the presence or absence of 10^{-3} M AMP ($s_{20,w}$ = 13.8 S) (Figure 6a), was found to be ultracentrifugally indistinguishable from native enzyme ($s_{20,w}$ = 13.6 S). In 0.2 M $NaClO_4$ (Figure 6b, lower curve) the enzyme appears to exist in equilibrating tetrameric and dimeric forms ($s_{20,w}$ = 13.5 and 8.7 S), as similarly observed in 2.5 M NaCl (Wang and Graves, 1963). Addition of 10^{-3} M AMP resulted in complete reversal of the changes in sedimentation (Figure 6b, upper curve; $s_{20,w}$ = 13.5 S), whereas addition of glucose-1-P at 0.032 M had no apparent effect on sedimentation of enzyme in 0.2 M $NaClO_4$.

Discussion

It is well recognized that the difference in enzymic and physical properties of phosphorylases a and b arise through the single difference in their primary structures, *viz.*, the presence of two phosphorylated seryl residues/250,000 g of phosphorylase a. It is clear that the addition of the ionic phosphate groups that occurs in the conversion of phosphorylase b to phosphorylase a must result in an alteration of the

protein structure so as to permit catalytic activity in the absence of AMP. It is therefore attractive to relate the effects of various salts reported in this paper to the effect of the covalently bound phosphate groups on enzyme structure and activity.

A concept to explain our results has been derived from the studies of Robinson and Jencks (1965) on the solubility of ATGEE, a model for peptide and amide groups in protein. Their work, and that of Nagy and Jencks (1965), showed that various salts could be ordered in a single series according to any of the three criteria, effect on the solubility of AGTEE, inhibition of protein denaturation, and promotion of polymerization of F-actin. For example, F^-, CH_3COO^-, and HPO_4^{2-}, which increase the activity coefficient of ATGEE, *i.e.*, salt out, are ineffective in depolymerizing F-actin even at 1.4–2.0 M, while ClO_4^-, which increases the solubility of ATGEE, brings about 50% depolymerization of F-actin at 0.35 M. These data suggest to us that activation of phosphorylase which occurs by phosphorylation and the effect of salts on phosphorylases b and a are due to an interaction of the covalently bound phosphate or salts with a specific protein site, located at or near the surface of the molecule, which is particularly sensitive to the ionic character of its environment. These interactions result in changes in activity coefficients of residues in this local region which in turn result in different enzyme conformations with different catalytic and physical properties. The notion that phosphorylase activity is influenced by interaction of salts at a specific site is supported by the recent findings of S. A. Mann and D. J. Graves (unpublished results) which show that limited tryptic attack of phosphorylase a which removes a hexapeptide containing the phosphorylated site (Fischer *et al.*, 1959) yields a form of the enzyme, phosphorylase b′, which is still active (Keller, 1955) but is no longer stimulated by fluoride. The significance of this hexapeptide to the proposed ion interaction site is currently under investigation.

Several differences between phosphorylases b and a can be explained by this model and are consistent with the observations of Robinson and Jencks (1965) and Nagy and Jencks (1965) on the effects of phosphate on protein stability, solubility, and polymerization. For example, it is recognized that phosphorylase a is more stable to thermal denaturation (Graves *et al.*, 1965), is more insoluble in water (Green, 1945), and dimerizes readily (Krebs and Fischer, 1956).

According to the model, the interactions of the anions F^- and ClO_4^- with the postulated ion interaction site affect catalytic activity and structure by the same mechanism as covalently bound phosphate, the direction of the effect depending on the particular anion. Thus, in the presence of F^-, the activity coefficients of residues at this site are so changed as to reduce the requirement for AMP and enhance the dimerization of the protein in the presence of AMP (phosphorylase b) or to reduce the extent of additional activation by AMP without change of the native tetrameric state of the protein (phosphorylase a). In all these particulars, ClO_4^- has the opposite effect, in accord with its opposite effect on the activity coefficient of ATGEE.

The kinetics of phosphorylase b with respect to AMP and the kinetics of phosphorylase a with respect to glucose-1-P in certain salt solutions did not fit the Michaelis–Menten relationship. Interestingly, in all these cases ultracentrifugal analysis showed the presence of two sedimenting components or indicated inhomogeneity. Since the two sedimenting species appear to correspond to dimeric and tetrameric forms of phosphorylases b and a, which were previously shown to have different enzymic activity (Wang and Graves, 1964; Wang *et al.*, 1965), nonlinear reciprocal plots are possibly related to mixtures of these forms. Under conditions where the kinetics followed the Michaelis–Menten relationship, little or no second component could be detected in the ultracentrifuge.

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Mammalian α-Acetylglucosaminidase. Enzymic Properties, Tissue Distribution, and Intracellular Localization*

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ABSTRACT: A preparation of α-acetylglucosaminidase, purified some 80-fold in low yield from extracts of pig liver, was almost devoid of α-acetylgalactosaminidase or any other known mammalian glycosidase. Both this partially purified enzyme and a crude extract manifested simple enzyme kinetics with the aryl glycosides used as test substrates. Inhibition effects were investigated.

The enzyme occurred at low levels of activity in all rat tissues examined. Experiments with rat liver homogenates characterized α-acetylglucosaminidase as a typical lysosomal acid hydrolase. Drastic disruption of lysosomes rendered this and three other lysosomal enzymes nonsedimentable only in part. An endogenous inhibitor of lysosomal hydrolases appeared to be present in the disrupted lysosomes.

α-Acetylglucosaminidase (α-2-acetamido-2-deoxy-D-glucoside acetamidodeoxyglucohydrolase, EC 3.2.1.-)[1] activity was first demonstrated in extracts of snail hepatopancreas (Zechmeister *et al.*, 1939). A similar enzymic activity was subsequently found to be present in mammalian tissues and was characterized more fully (Roseman and Dorfman, 1951). The levels of activity in the sources described were extremely low, as measured with phenyl α-*N*-acetylglucosaminide, the test substrate used (see also, Findlay *et al.*, 1958; Watkins, 1959). Concentration of the mammalian enzyme has now been undertaken, with the intention of examining its specificity and of discovering the nature of its endogenous substrates, if possible. Reported here is some information regarding the localization and properties of the enzyme, which was gathered incidental to work on its purification, still in progress. For convenience of presentation, there have also been included certain parallel observations relating to α-acetylgalactosaminidase (α-2-acetamido-2-deoxy-D-galactoside acetamidodeoxygalactohydrolase, EC 3.2.1.-), a distinct mammalian enzyme, devoid of α-acetylglucosaminidase activity (Weissmann and Friederici, 1966), which was detected in the course of these experiments.

Experimental Procedures

Materials. *p*-Nitrophenyl α- and β-D-glucopyranosides, *p*-nitrophenyl β-D-galactopyranoside, *p*-nitrophenyl α-D-mannopyranoside, *p*-nitrophenyl α-L-fucopyranoside, and *p*-nitrophenyl β-D-xylopyranoside were obtained from the Pierce Chemical Co. Phenolphthalein glucuronide was obtained from the Sigma Chemical Co. The phenyl 2-acetamido-2-deoxy-α- and β-mannopyranosides were prepared by an extension, to be published, of methods used previously (Weissmann, 1966). The other glycosidase substrates used have been described (Weissmann, 1966). *N*-Acetylglucosaminolactone was prepared as an amorphous solid (Findlay *et al.*, 1958). Folin–Ciocalteau reagent (2 N) was purchased from the Fisher Scientific Co. and the nonionic detergent "Triton X-100" from the Rohm and Haas Co. Triethylaminoethylcellulose (TEAE-cellulose, 0.44 mequiv/g) was purchased from

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[1] Those enzymes not identified by IUB systematic nomenclature or number in the text are so identified in Table IV.