Nonenzymatic Acetylation of Histones with Acetyl Phosphate and Acetyl Adenylate†

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ABSTRACT: Nonenzymatic acetylation of calf-thymus lysine- and arginine-rich histones was demonstrated to occur when these proteins were incubated with [^{14}C]acetyl phosphate and [^{14}C]acetyl adenylate. The levels of acetylation depend on both pH and on reagent concentration. When acetyl [^{33}P]phosphate and acetyl [^{3}H]adenylate were used as reagents, we found neither histone phosphorylation nor adenylylation. Most of the radioactivity of ^{14}C-labeled acetylated histones was recovered as N^{ϵ}-acetyllysine. Furthermore, only a small amount of O-bound radioactivity was released by the ^{14}C-labeled acetylated arginine-rich histone during treatment with hydroxylamine. Experiments on the acetylation of histones, in the presence of increasing salt concentration, gave different results for the two acetylating agents.

The presence of *N*-acetyl groups in calf-thymus histones was first reported by Phillips (1963). Phillips (1968) also showed that the NH_2 terminal of several histone fractions was acetylated. However, subsequent studies on isolated calf-thymus nuclei revealed that the major site of acetylation in histones is not the NH_2-terminal amino acid, but rather the ϵ-amino group of internal residues (Gershey et al., 1968; Vidali et al., 1968). Recently the location of sites of in vivo acetylation of trout testis histones was studied (Candido and Dixon, 1971, 1972).

The mechanism of histone acetylation which has been the most thoroughly studied is the enzymatic one, in which acetyl-CoA is the acetyl donor, although it has been postulated that above pH 7.5 acetylation proceeds through a chemical mechanism (Gallwitz, 1968). This has been clearly demonstrated by Paik et al. (1970), who found an optimum pH of 10.0 for the chemical acetylation of histones by acetyl-CoA.

In previous works (Ramponi et al., 1970; Ramponi and Grisolia, 1970) it was demonstrated that carbamyl phosphate and 1,3-diphosphoglycerate react rapidly with histones.

In the present paper we have performed an extension of the study on the modification of histones by two other physiological compounds in which a carboxyl phosphate bond is present, i.e., acetyl phosphate and acetyl adenylate.[1]

Materials and Methods

Preparation of Acetyl Phosphate, Acetyl Adenylate, and Acetyl Ribosyl Phosphate. Acetyl phosphate (Kornberg et al., 1956) was prepared by the following method: 0.95 ml of pyridine, 0.5 ml of 1 *M* K_2HPO_4 (^{33}P labeled to have [^{33}P]Ac-P), and 1.5 ml of water were mixed and cooled to 0°. Acetic anhydride (0.11 ml) (^{14}C labeled to have [^{14}C]Ac-P) was added over a 3-min period to the mixture which was then stirred. Then 0.45 ml of 4 *N* LiOH was added, and the product precipitated with 23 ml of ethanol (−15°). Successively the precipitate was collected, washed twice in cold ethanol, and dried in vacuo.

Acetyl adenylate (Jencks, 1963) was prepared by the following method: 2 mmol of 5′-AMP ([^{3}H]adenosine labeled to have Ac-[^{3}H]AMP), dissolved in 10 ml of 50% pyridine, was mixed with 2 ml of 1 *M* LiOH and cooled to 0°. Then 2 ml of acetic anhydride (^{14}C labeled to have [^{14}C]Ac-AMP) was added over 2–3 min with stirring, and the mixture immediately extracted three times with cold ether. The aqueous layer was poured drop by drop into 150 ml of cold acetone, with stirring. Successively the precipitate was collected, dissolved in a small amount of water, and again precipitated with cold acetone. The product was left to dry overnight under vacuum.

Acetyl ribosyl phosphate was prepared by the following method: [^{14}C]acetyl ribosyl phosphate was synthesized (with slight modification) by the synthesis reported above for Ac-AMP, using ribose 5-phosphate instead of AMP as starting material. The acetone-precipitated product was an oil and did not crystallize. The oil was dissolved in a small amount of water and stored at −80°. The product, analyzed for carboxyl phosphate content (the Lipmann and Tuttle method, 1945) and for radioactivity, showed a molar ratio of [^{14}C]acetyl:carboxyl phosphate of 1.07, demonstrating that only one acetyl group is bound to ribose 5-phosphate.

Acetylation Experiments. Acetylation experiments were carried out by incubating 2 mg of histones (calf-thymus arginine and lysine-rich histones, types IV and III, respectively, from Sigma) or other proteins with [^{14}C]Ac-P or [^{14}C]Ac-AMP (the amount is specified in each experiment) in a total volume of 0.32 ml at 37° for suitable periods of time. Then the mixtures were acidified by the addition of a few drops of concentrated acetic acid and applied to 1 × 30 cm Sephadex G-25 columns, equilibrated with 0.1 *M* KCl, and eluted at a flow rate of 8 ml/hr (four to eight of these columns were used simultaneously). One-milliliter fractions were collected and transferred to a counting vial; then 10 ml of scintillation solution [⅓ part of Triton X-100 and ⅔ parts of Liquiflor (NEN Chem. GmbH) diluted 1:10 with toluene] was added. Radioactivity was measured by an Isocap-

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[1] Abbreviations used are: Ac-P, acetyl phosphate; Ac-AMP, acetyl adenylate.

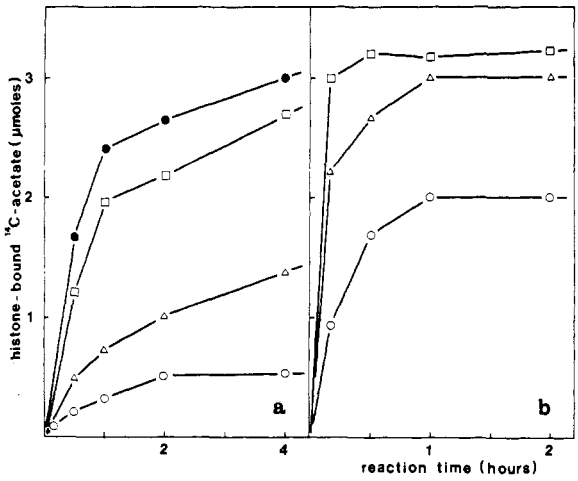

FIGURE 1: Lysine-rich histone acetylation by Ac-P (a) and Ac-AMP (b) vs. incubation time. The reaction mixture was as follows: 2 mg of histone, 40 μmol of buffer, 20 μmol of Ac-P, or 5 μmol of Ac-AMP in a total volume of 0.32 ml. The incubation was performed at 37° for varying lengths of time: (○) Tris-HCl buffer (pH 7.6); (△) Tris-HCl buffer (pH 8.9); (□) Borate buffer (pH 10.0); (●) borate buffer (pH 11.0). No significant differences were found using borate or Tris-HCl buffer at the same pH value.

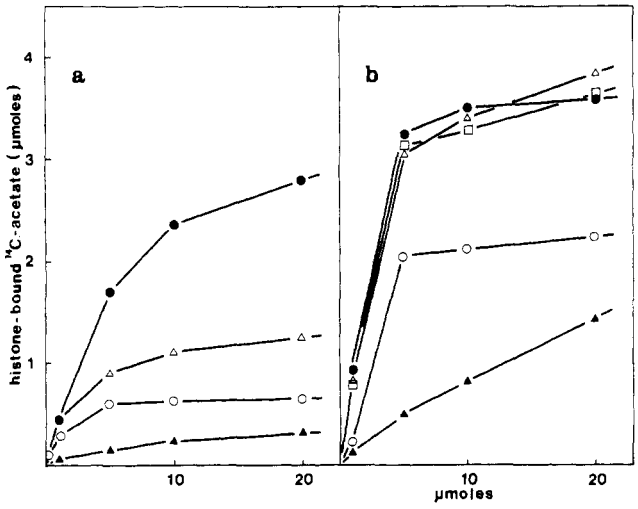

FIGURE 2: Lysine-rich histone acetylation by Ac-P (a) and Ac-AMP (b) vs. reagents concentration. The reaction mixture was as follows: 2 mg of histone, 40 μmol of buffer, and varying amounts of Ac-P or Ac-AMP in a total volume of 0.32 ml. The incubation times at 37° were 3 and 1 hr, for Ac-P or Ac-AMP, respectively: (▲) acetate buffer (pH 5.3); (○) Tris-HCl buffer (pH 7.6); (△) Tris-HCl buffer (pH 8.9); (□) borate buffer (pH 10.0); (●) borate buffer (pH 11.0).

300 liquid scintillation counter.

Two well-separated radioactive peaks were found, the first corresponding to high molecular weight material and the second to low molecular weight material. The first peak radioactivity was taken to be histone bound.

In order to dissolve the precipitate formed during reaction in arginine-rich histone experiments, solid urea and some drops of concentrated acetic acid were added before applying to the columns of Sephadex G-25, in 0.1 *M* KCl containing 6 *M* urea.

Enzymic Hydrolysis. Native or ^{14}C-labeled acetylated histones were submitted to enzymic hydrolysis according to Hill and Schmidt (1962), slightly modified by Ramponi et al. (1971). Aliquots of the deproteinized hydrolysates were directly applied to the columns of a Unichrom amino acid analyzer. To measure radioactivity, the exit of the Unichrom photometer was connected to a fraction collector. Aliquots of each fraction were put into a counting vial, mixed with Triton X-100–Liquiflor solution, and counted.

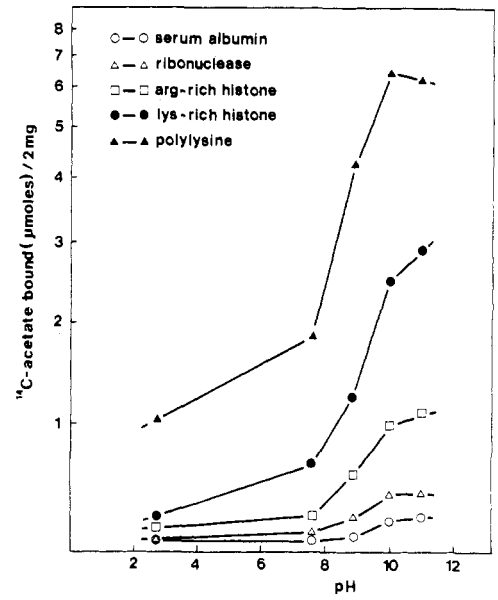

FIGURE 3: The acetylation of some proteins and polylysine by Ac-P as a function of pH. The reaction mixture, in a total volume of 0.32 ml, contained 2 mg of proteins or polylysine, 40 μmol of buffer at various pH values, and 20 μmol of Ac-P, and was incubated at 37° for 3 hr.

Results and Discussion

Effect of Incubation Time, Substrate Concentration, and pH on Lysine-Rich Histone Acetylation. To study the acetylation behavior of lysine-rich histone by Ac-P and Ac-AMP, we carried out experiments in which 2 mg of histone was incubated with a fixed amount of acetylating reagent for varying lengths of time at various pH values. The results are reported in Figures 1a and 1b. It can be noted that reactions are dependent on the incubation period, and reaction rate is faster with Ac-AMP than it is with Ac-P.

Furthermore, Figures 2a and 2b show that the degree of acetylation depends on the concentration of the reagents for both Ac-P and Ac-AMP, and that the behavior of the curves is similar. An exception is the reaction of histone with Ac-AMP at pH 5.3, where a plateau is not approached even at about 60 m*M* Ac-AMP concentration. Figures 1 and 2 also show the marked pH dependence of both these reactions.

From Figure 2b it can be seen that when lysine-rich histone is incubated with 1 μmol of Ac-AMP, at pH values 8–11, most of the acetate is incorporated into the protein. This fact, together with the hydrolysis rate values of Ac-AMP (half-life times of 17 and 2 min at pH 9 and 10, respectively, as measured at 37° by a pH-Stat apparatus), suggest that the rate of the histone–Ac-AMP reaction is very high at a low Ac-AMP concentration.

Acetylation Specificity. Figure 3 shows the radioactive labeling of some proteins and synthetic polylysine (Sigma Chemical Co.), incubated with [^{14}C]Ac-P at various pH values. It can be noted that, while polylysine and lysine-rich histone were highly acetylated, arginine-rich histone was acetylated to a lesser degree. A small amount of radioactivity was seen to be bound in other proteins tested, i.e., albumin and ribonuclease. Nevertheless, the lysine content of these last two proteins is similar to that of arginine-rich histone. The above results may be explained by considering

that albumin and ribonuclease have a more highly ordered structure than of the randomly coiled polylysine and histones. Thus, these native proteins have a smaller number of exposed lysine ϵ-NH_2 groups on their molecular surfaces than do histones.

On the Possibility of Phosphorylation or Adenylylation of Histones by Ac-P and Ac-AMP. To check this possibility, we carried out experiments in which [^{33}P]Ac-P and Ac-[^{3}H]AMP were incubated with lysine-rich and arginine-rich histones under the same conditions as those for acetylation experiments, at pH values of 2.7, 7.6, and 8.9. The histones were isolated from reagents as before. No radioactivity, ^{33}P or ^{3}H, respectively, was found to be histone bound at all pH values tested. From these data we can conclude that the only apparent reaction between Ac-P or Ac-AMP and histones is the nucleophilic attack at the carbonyl carbon of the acetyl group.

Effect of Various Compounds on Lysine-Rich Histone Acetylation. In these experiments, carried out at pH 8.9, we tested the competition power of various compounds having different chemical groups on the acetylation of lysine-rich histone by Ac-P or Ac-AMP. The results are reported in Figure 4; it can be seen that 1-propanol, 2-mercaptoethanol, and N^{α}-acetyl-L-arginine (synthesized according to Greenstein and Winitz, 1961) have little or no effect on either of the two reactions. Furthermore, while *p*-oxybenzoic acid is a good competitor in Ac-P reactions, its effect on the Ac-AMP reaction is minimal. This can be explained if we consider the steric hindrance of the benzene ring and the structural differences between Ac-P and Ac-AMP.

The different behavior of alanine may be explained by considering that, at pH 8.9, the two carboxyl phosphate compounds exist practically as dianion (Ac-P^{2-}) and monoanion (Ac-AMP^{-}), respectively. Alanine, whose better reactive form at this pH is $CH_3CHNH_2COO^{-}$, is probably repelled by the two negative charges of Ac-P^{2-}. The reduced negative charge of Ac-AMP^{-}, combined with the major reactivity of this last compound, facilitates the nucleophilic attack by the α-NH_2 nitrogen of alanine. This fact is in agreement with the apparently greater competition power of lysine against Ac-AMP than against Ac-P reaction.

From the above competition experiments it is possible to suggest that (1) under certain conditions, tyrosine could be O-acetylated by Ac-P; (2) α-NH_2 groups can be more easily acetylated by Ac-AMP than by Ac-P.

Identification of [^{14}C]Acetyl-Labeled Amino Acid(s). To identify the amino acid(s) of histones, acetylated either by Ac-P or by Ac-AMP, complete enzymic hydrolysis and amino acid analyses were carried out. Only one radioactive and ninhydrin-positive peak was found, which appears between proline and glycine. Its elution time corresponds exactly to that of synthetic N^{ϵ}-acetyllysine (Cyclo Chemical Co.). The radioactive material, isolated by a preparative run, was subsequently hydrolyzed with 6 *N* HCl at 110° for 24 hr, and the amino acid analysis performed on this hydrolysate gave only lysine.

Only for arginine-rich histone (acetylated by Ac-AMP) was a small amount of radioactivity (this material is not ninhydrin positive) eluted before aspartic acid. This could be explained by the presence of free acetic acid, partially split off from *N*- or *O*-acetyl groups of particular lability during the enzymic hydrolysis. Thus, further experiments were made to check if O-acetylated sites could be present in acetylated histones, according to the method of Narita (1959), with slight modification. Each acetylated histone (about 4 mg) was treated with 2 *M* hydroxylamine, adjusted to pH 6.5, for 20 min at 37°, and then introduced into a column of Sephadex G-25 (1 × 40 cm) and eluted with 0.1 *M* KCl. Fractions (0.8–1 ml) were collected and their radioactivity measured. The radioactivity found in the peak of low molecular weight material was taken to be O bound. Only acetylated arginine-rich histone released O-bound radioactivity (7 and 12% for Ac-P and Ac-AMP, respectively). This fact is similar to that found in enzymatically acetylated histones as reported by Nohara et al. (1966), who also observed hydroxylamine acetyl stability for lysine-rich histone.

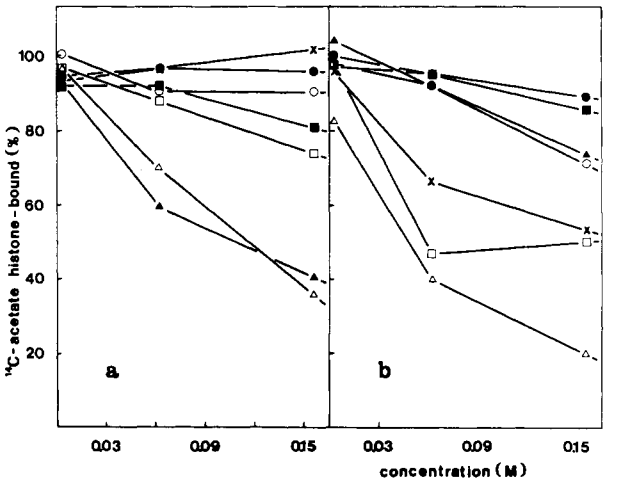

FIGURE 4: Competition of various compounds on lysine-rich histone acetylation by Ac-P (a) and Ac-AMP (b). The reaction mixtures, in a total volume of 0.32 ml, incubated for 3 or 1 hr at 37° for Ac-P (5 μmol) or Ac-AMP (5 μmol), respectively, contained 2 mg of histone, 40 μmol of Tris-HCl buffer (pH 8.9), and various amounts of the following compounds: (○) 2-mercaptoethanol; (△) lysine; (□) alanine; (●) 1-propanol; (▲) *p*-oxybenzoic acid; (■) N^{α}-acetyl-L-arginine; (×) imidazole.

In Table I are summarized the quantitative results of the amino acid analysis of enzymic hydrolysates. It is possible to observe that lysine content is remarkably lowered in acetylated histones with the contemporaneous appearance of N^{ϵ}-acetyllysine, while the amount of other amino acids remains similar.

Effect of Salt Concentration on Histone Acetylation with Ac-P and Ac-AMP. The purpose of these experiments was to show the behavior of histone acetylation in a solution of increasing ionic strength. This is because histones undergo conformational changes in salt solutions (Bradbury and Crane-Robinson, 1971; Hnilica, 1972) and it was found that in chromatin the conformation of histones approaches that of these proteins in a solution of high ionic strength (Tuan and Bonner, 1969). This type of experiment may be utilized in probing histone structure in chromatin.

Figure 5 shows histone acetylation at different concentrations of salts. The Ac-P reaction for both lysine- and arginine-rich histones is markedly decreased by the increased concentration of salt. On the other hand, the concentration of salt has no effect on the reaction between Ac-AMP and both histones. As regards preincubation, no differences were observed in the experiments carried out, either with or without preincubation of histones with salts, except in the case of acetylation of arginine-rich histone with Ac-AMP (Figure 5d). In this figure it can be seen that preincubation causes a more extensive acetylation of histone.

Table I: Amino Acid Analyses of Histones' Enzymatic Hydrolysates.[a]

Amino Acid	Lys-Rich His	Ac-P Acetylated Lys-Rich His	Ac-AMP Acetylated Lys-Rich His	Arg-Rich His	Ac-P Acetylated Arg-Rich His	Ac-AMP Acetylated Arg-Rich His
Asp	1.3	1.3	1.3	3.1	4.1	3.6
Thr	6.0	6.1	6.7	6.6	6.4	6.1
Ser + amides	7.7	7.3	7.6	6.1	5.4	5.5
Glu	3.8	3.5	3.3	6.4	7.4	6.5
Pro	7.0	6.8	6.7	3.9	4.3	4.5
N^{ϵ}-Ac-Lys		17.1	25.7		4.9	12.4
Gly	7.3	7.3	7.4	8.7	8.7	8.2
Ala	27.5	26.8	24.8	12.2	12.5	12.2
Half-Cys				0.3		
Val	4.7	4.6	4.5	6.0	6.0	5.8
Met	0.1	0.1	0.1	1.2	0.7	1.4
Ile	1.0	1.0	1.0	5.2	5.1	5.0
Leu	4.5	4.4	4.4	7.9	7.8	7.8
Tyr	0.7	0.6	0.7	3.1	2.9	3.1
Phe	0.7	0.6	0.5	2.4	2.4	2.6
Lys	25.0	10.2	2.3	13.1	8.3	2.4
His	0.2	0.1	0.1	1.8	1.7	1.9
Arg	2.0	1.9	2.6	11.5	11.2	10.8

[a] Results are expressed as mol/100 mol of total amino acids. Histones' acetylation was carried out at pH 8.9.

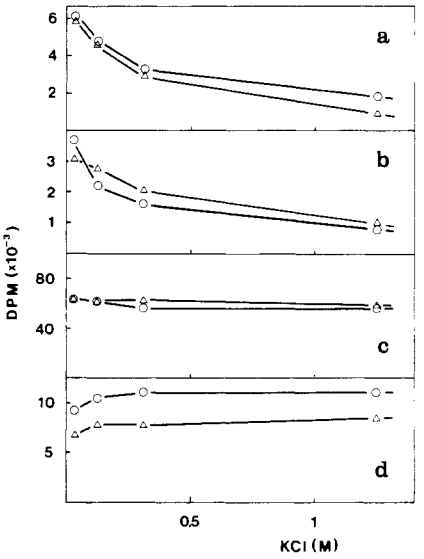

FIGURE 5: Effect of salt concentration on histone acetylation by Ac-P and Ac-AMP. (a) Ac-P acetylation of lysine-rich histone. Incubation mixture (37° for 3 hr) contained: 2 mg of histone, 40 μmol of Tris-HCl buffer (pH 8.9), 5 μmol of Ac-P, and various amounts of KCl. (b) Ac-P acetylation of arginine-rich histone. The experimental conditions are as in a. (c) Ac-AMP acetylation of lysine-rich histone. Reaction mixture (37° for 1 hr) contained: 2 mg of histone, 40 μmol of Tris-HCl buffer (pH 8.9), 5 μmol of Ac-AMP, and various amounts of KCl. (d) Ac-AMP acetylation of arginine-rich histone. Experimental conditions are as in c. (○) The mixture, containing only histones, buffer, and salt, was preincubated 3 hr before adding an acetylating reagent dissolved in a salt solution with the same KCl concentration as the preincubation mixture; (△) without preincubation.

In experiments with salts the different behavior of the two carboxyl phosphate compounds, i.e., Ac-P and Ac-AMP, can be related to their mono- or dianionic forms, respectively. This hypothesis is supported by the results obtained with acetyl ribosyl phosphate as acetylating agent. When this compound is used in histone acetylation, salts have no effect, as they do for Ac-AMP.

The results reported in this paper demonstrate that Ac-P and, more rapidly, Ac-AMP react nonenzymatically with histones and other proteins in a broad pH range and also at low substrate concentration.

For this type of reaction, in which a substrate usually reacts nonspecifically with an enzyme or another protein, forming a covalent bond and inducing a molecular distortion, the term *chemotrophic* was proposed (Grisolia and Hood, 1972).

In a recent review Grisolia and Hood (1972) described many examples of these phenomena and postulated a physiological relevance of the *chemotrophic* modification of proteins.

As regards this paper, it can be noted that Ac-AMP, in particular, is a model for numerous compounds which are synthesized in high quantity in the cell, such as aminoacyl adenylates or fatty acyl adenylates, related to carboxyl activation in protein synthesis and in fatty acid metabolism, respectively. If the reactions described above have some physiological relevance, it can be considered that enzymes which hydrolyze acyl adenylates (Kellerman, 1958) and acyl phosphates (Lipmann, 1946) were found. These enzymes could play a regulatory role in physiological concentration of these highly reactive compounds. We found that horse muscle acyl phosphatase (Ramponi et al., 1969), for example, protects lysine-rich histone from acetylation when it is added to a mixture containing histone and Ac-P.

References

Bradbury, E. M., and Crane-Robinson, C. (1971), in Histones and Nucleohistones, Phillips, D. M. P., Ed., New York, N.Y., Plenum Publishing Co., p 85.

Candido, E. P. M., and Dixon, G. H. (1971), *J. Biol. Chem. 246,* 3182.

Candido, E. P. M., and Dixon, G. H. (1972), *Proc. Natl. Acad. Sci. U.S.A. 69,* 2015.

Gallwitz, D. (1968), *Biochem. Biophys. Res. Commun. 32,* 117.

Gershey, E. L., Vidali, G., and Allfrey, V. G. (1968), *J. Biol. Chem. 243,* 5018.

Greenstein, J. P., and Winitz, M. (1961), in Chemistry of the Amino Acids, New York, N.Y., Wiley, p 1850.

Grisolia, S., and Hood, W. (1972), in Biochemical Regulatory Mechanism in Eukaryotic Cells, Kun, E., and Griso-

lia, S., Ed., New York, N.Y., Wiley-Interscience, p 137.
Hill, R. L., and Schmidt, W. R. (1962), *J. Biol. Chem. 237,* 389.
Hnilica, L. S. (1972), in The Structure and Biological Function of Histones, Cleveland, Ohio, CRC Press, p 49.
Jencks, W. P. (1963), *Methods Enzymol. 6,* 762.
Kellerman, G. M. (1958), *J. Biol. Chem. 231,* 427.
Kornberg, A., Kornberg, S. R., and Simms, E. S. (1956), *Biochim. Biophys. Acta 20,* 215.
Lipmann, F. (1946), *Adv. Enzymol. Relat. Subj. Biochem. 6,* 231.
Lipmann, F., and Tuttle, L. C. (1945), *J. Biol. Chem. 159,* 21.
Narita, K. (1959), *J. Am. Chem. Soc. 81,* 1751.
Nohara, H., Takahashi, T., and Ogata, K. (1966), *Biochim. Biophys. Acta 127,* 282.
Paik, W. K., Pearson, D., Lee, H. W., and Kim, S. (1970), *Biochim. Biophys. Acta 213,* 513.
Phillips, D. M. P. (1963), *Biochem. J. 87,* 258.
Phillips, D. M. P. (1968), *Biochem. J. 107,* 135.
Ramponi, G., Cappugi, G., Treves, C., and Nassi, P. (1971), *Life Sci. 10,* 983.
Ramponi, G., and Grisolia, S. (1970), *Biochem. Biophys. Res. Commun. 38,* 1056.
Ramponi, G., Grisolia, S., and Ramponi, G. (1970), Fed. Amer. Soc. Expt. Biol., 54th Annual Meeting, Atlantic City, 2775.
Ramponi, G., Guerritore, A., Treves, C., Nassi, P., and Baccari, V. (1969), *Arch. Biochem. Biophys. 130,* 362.
Tuan, D. Y. H., and Bonner, J. (1969), *J. Mol. Biol. 45,* 59.
Vidali, G., Gershey, E. L., and Allfrey, V. G. (1968), *J. Biol. Chem. 243,* 6361.

Isolation and Characterization of Subunits from the Predominant Form of *Dolichos biflorus* Lectin†

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ABSTRACT: The subunits of the two molecular forms (A and B) of the *Dolichos biflorus* lectin were isolated by ion-exchange chromatography on DEAE-cellulose in 8.0 *M* urea. Subunits IA and IIA which comprise the predominant molecular form A of the lectin were found to have molecular weights of 27,700 and 27,300, respectively, as determined by sedimentation equilibrium studies in 8.0 *M* urea. These subunits have similar amino acid compositions and each have alanine at their amino-terminal ends. Comparison of the IA and IIA subunits by immunodiffusion against antisera to the seed extract as well as to subunits IA and IIA showed no antigenic differences between the two subunits. Carboxyl terminal analyses of subunits IA and IIA with carboxypeptidase A produced an essentially simultaneous release of both leucine and valine residues from subunit IA; no detectable amino acids were released from subunit IIA under identical conditions. The data suggest that the molecular form A of the lectin (molecular weight 113,000, Carter and Etzler, 1975) consists of four subunits with a possible stoichiometry of IA_2IIA_2. Other possible arrangements of the subunits are discussed.

The seeds of many plants contain proteins called lectins that have the ability to specifically agglutinate certain types of cells (for review see Lis and Sharon, 1973). A number of these lectins have specificities for some of the various blood group substances; among these lectins is the *Dolichos biflorus* lectin that was reported to agglutinate type A erythrocytes (Bird, 1951) and to precipitate blood group A substance (Boyd and Shapleigh, 1954; Bird, 1959). This lectin was isolated in a highly purified state by affinity chromatography on insoluble blood group A+H substance, and its blood group A specificity was found to be due to its ability to specifically combine with terminal nonreducing *N*-acetyl-α-D-galactosamine residues (Etzler and Kabat, 1970).

The isolated *Dolichos biflorus* lectin is a glycoprotein (Etzler and Kabat, 1970; Font et al., 1971) and has recently been fractionated into two electrophoretically distinguishable forms (A and B) by chromatography on concanavalin A-Sepharose. These two molecular forms of the lectin have differences in carbohydrate contents but have identical specificities and very similar amino acid compositions (Carter and Etzler, 1975).

The A and B forms of the lectin have molecular weights of 113,000 and 109,000, respectively, and are each dissociated into two types of subunits by discontinuous electrophoresis on sodium dodecyl sulfate–urea gels (Carter and Etzler, 1975).

In the present paper we report the isolation of the subunits of the *Dolichos biflorus* lectin and the characterization of the two types of subunits of the predominant form A of the lectin.

Materials and Methods

Isolation of Lectin. The *Dolichos biflorus* lectin was isolated from seed extracts as previously described (Etzler and Kabat, 1970; Etzler, 1972) by adsorption onto insoluble polyleucyl hog blood group A+H substance (Kaplan and Kabat, 1966) and specific elution from this immunoadsorbent with 0.01 *M* *N*-acetyl-D-galactosamine. After removal of the hapten by chromatography on Bio-Gel P-10, the lectin was concentrated by ultrafiltration in a Diaflo ultrafil-

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