hibitory action of a proton is reinforced by the dicarboxylic acid. Under certain conditions even the keto acid substrates may act as inhibitors rather than activators of the transamination.[8]

The spectral changes with *p*H may be interpreted as follows. In terms of the studies of Metzler and Snell[3,9] the forms of the pyridoxal imine which may affect the spectra are shown in Fig. 2.

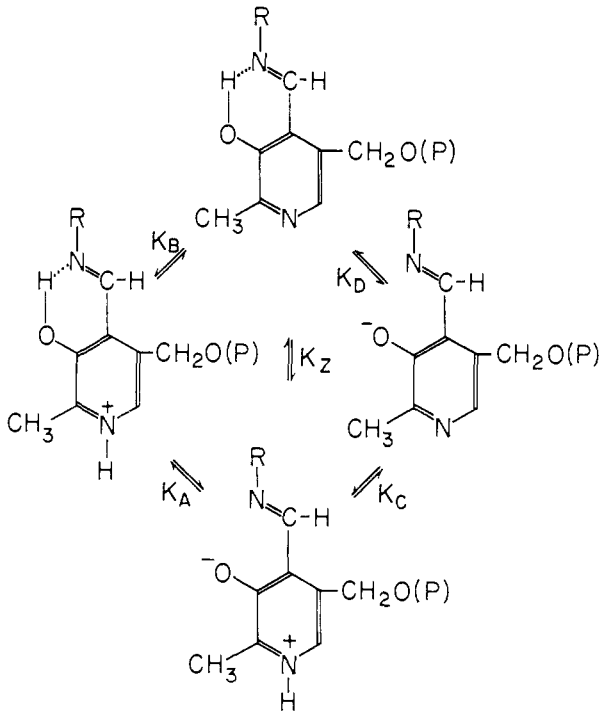

Fig. 2.—The ionization of pyridoxal phosphate imines.

These equations are based on the assumption that the yellow color may be ascribed to the hydrogen chelate ring. Rupture of this ring by a change in *p*H results in loss of color. A pK_A of 6.2 for this color change for the transaminase suggests that it is the dipolar form of the imine which occurs at neutrality, in contrast to the non-polar forms of the imines studied by Metzler[3] (pK_B = 5.9, pK_D = 10.5).

The implications of these findings with reference to the mechanism of enzymatic transamination will be discussed elsewhere.

Acknowledgment.—We are happy to acknowledge financial support from Ethicon, Inc.

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(9) D. E. Metzler and E. E. Snell, THIS JOURNAL, **77**, 2431 (1955).

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A STABLE EQUIMOLAR COPPER(II)-ALBUMIN COMPLEX

Sir:

A simple and rapid amperometric titration technique has been developed for the determination of bovine serum albumin (BSA) with copper(II) in ammoniacal buffer at *p*H 9.2. Figure 1 shows current voltage curves of copper(II) at the rotated platinum electrode (RPE) in a buffer 0.1 *M* in ammonia and 0.1 *M* in ammonium nitrate (curve B). The first wave corresponds to the reduction of copper(II) to copper(I). No copper waves are observed when albumin is present in a molar concentration greater than that of copper (curve D). Curve E illustrates the reappearance of the copper waves when an excess of copper is present.

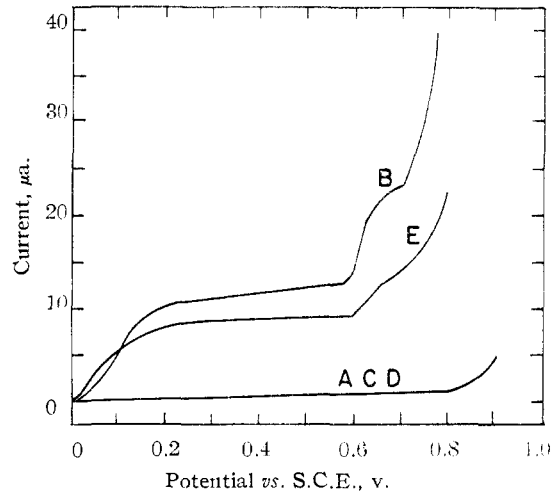

Fig. 1.—Current voltage curves at rotated platinum electrode; buffer, 0.1 *M* NH_3 + 0.1 *M* NH_4NO_3; *p*H 9.2; speed of rotation, 900 r.p.m.: A, residual; B, 4.0 × 10^{-5} *M* Cu(II); C, 7.3 × 10^{-5} *M* BSA; D, 7.3 × 10^{-5} *M* BSA + 6.5 × 10^{-5} *M* Cu(II); E, 7.3 × 10^{-5} *M* BSA + 13.0 × 10^{-5} *M* Cu(II).

By amperometric titration at the RPE in ammonia buffer at −0.4 volt, we have found that copper(II) reacts rapidly with native BSA in a mole ratio of one to one. Similar results have been obtained under proper conditions at *p*H 8 and 10. The rapid reaction is followed by a slower reaction of additional amounts of copper(II); this does not interfere with the titration. The sulfhydryl group is not involved in the reaction with copper(II). After addition of one or two moles of copper(II) per mole BSA, 0.68 mole sulfhydryl per mole BSA[1] was found by subsequent amperometric titration with mercuric chloride and/or silver nitrate.[2] Also, addition of 0.68 mole of silver nitrate or mercuric chloride per mole BSA prior to titration with copper(II) did not affect the results of this titration.

Titrations of BSA have also been carried out in a denaturing mixture which was 4 *M* in guanidine hydrochloride, 0.1 *M* in ammonia and 0.1 *M* in ammonium nitrate. A reaction ratio of copper(II) to BSA somewhat greater than unity (about 1.3) was found. This higher reaction ratio is ascribed to oxidation by copper(II)[3] of the sulfhydryl group which is oxidizable to disulfide in denatured al-

(1) W. L. Hughes, Jr., THIS JOURNAL, **69**, 1836 (1947); *Cold Spring Harbor Symposium Quant. Biol.*, **14**, 79 (1949).

(2) I. M. Kolthoff, W. Stricks and L. Morren, *Anal. Chem.*, **26**, 366 (1954); I. M. Kolthoff and W. Stricks, THIS JOURNAL, **72**, 1952 (1950).

(3) I. M. Kolthoff and W. Stricks, *Anal. Chem.*, **23**, 763 (1951).

bumin. When the sulfhydryl group in denaturing medium is inactivated by addition of 0.68 mole of silver nitrate or potassium ferricyanide, the reaction ratio of copper(II) to BSA was again found to be unity.

Our experiments have been carried out under conditions quite different from those of Klotz, *et al.*,[4] and no comparison with their results is made here. Our results show that in the *p*H range 8–10, one copper(II) is bound to the BSA molecule extremely tightly, and that the sulfhydryl group is not the reactive group. It is of interest to note that nickel(II), even in very small concentrations, and also, to a lesser extent, cobalt(II) greatly interfere with the copper(II) BSA reaction while even large amounts of zinc(II) have very little effect. Thus, the group in the BSA molecule responsible for the binding of copper(II), the nature of which is at present unknown, apparently also forms stable complexes with nickel(II) and cobalt(II) but not with zinc(II). One unique site in the BSA molecule is the N-terminal aspartyl group, and further work may show whether this group is the site of copper(II) binding.

There is very little (if any) reaction between copper(II) and human γ-globulin under our conditions. The method has been applied to the determination of albumin in blood serum, and preliminary results are promising.

Acknowledgment.—This work was supported by grants from the U. S. Public Health Service and from the Louis and Maud Hill Family Foundation.

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ENZYME CATALYSIS AND ENZYME SPECIFICITY—COMBINATION OF AMINO ACIDS AT THE ACTIVE SITE OF PHOSPHOGLUCOMUTASE[1]

Sir:

In the classical template model for enzyme action the substrate is assumed to be absorbed with a precise fit into an area called the "active site" of the protein.[2] Presumably the amino acids at this site provide not only the catalytic action but also the specificity of the enzyme. From the widely varying specificity patterns of enzymes it would be anticipated that the amino acid composition at the active site would vary to fit the particular substrate. On the other hand, stereochemical and other evidence had led to the hypothesis that a few bond-breaking mechanisms might be common to reactions of widely different specificities[3] and hence there might be some common amino acid sequences. The sequence of a portion of the active site of chymotrypsin has been established[4–6] and hence a comparison could be made if similar information could be obtained for an enzyme of very different specificity. Such an opportunity arose when it was demonstrated that the active site of phosphoglucomutase was marked by a serine phosphate.[7,8]

P^{32}-labeled enzyme was prepared by exchange with radioactive substrates (glucose-1-PO_4^{32} and glucose-6-PO_4^{32}) under conditions similar to those of the standard assay.[9] It had been established that the enzyme is labeled only at the active site by this procedure.[8] The enzyme was then degraded, either with acid alone, with proteolytic enzymes alone, or with proteolytic enzymes followed by acid hydrolysis. Radioactive phosphopeptides were isolated by use of Dowex 50 columns[10,11] and paper chromatography. The compositions of the phosphopeptides are shown in Table I.

TABLE I

P^{32}-PHOSPHOPEPTIDES ISOLATED FROM PHOSPHOGLUCOMUTASE

Peptide	Phosphopeptide obtained by treatment with	Amino acid compn.
1	Proteolytic + acid	(Asp,Ser,Gly,Glu)
2	Proteolytic + acid	(Asp,Ser,Gly,Glu,Ala)
3	Proteolytic + acid	(Asp,Ser,Gly,Glu,Ala,Val,Thr)
4	Proteolytic only	(Asp,Ser,Gly,Glu)
5	Proteolytic only	(Asp,Ser,Gly,Glu Ala,Val,Thr, Leu)
6	Acid only	(Asp,Ser,Gly,Glu,Ala)
7	Acid only	(Asp,Ser,Gly,Glu,Ala,Val Thr, Leu)

While the detailed sequence has not been completely established, it is to be noted that the composition of each peptide and the order from comparison of lower and higher peptides is in perfect agreement with the sequence for chymotrypsin which is Asp Ser Gly Glu Ala Val.[4–6] The probability that the 7 peptides isolated from phosphoglucomutase would agree with this sequence by chance alone is very small. Moreover the concordance of the acid derived and proteolytic enzyme plus acid derived peptides is strong evidence against the sequence being an artifact of the separation procedure. The data therefore indicate that the amino acid sequence of the active site for at least 6 amino acids is the same for an enzyme which specifically hydrolyzes peptide bonds and an enzyme that specifically transfers phosphate between carbohydrate molecules.

This apparently surprising result is good support for the above mentioned hypothesis that common bond-breaking mechanisms exist despite vary-

(1) Research carried out at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.

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(5) N. K. Schaffer, S. Harshman, R. R. Engle and R. W. Drisko, *Federation Proc.*, **14**, 275 (1955).

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(11) N. K. Schaffer, S. Harshman and R. R. Engle, *ibid.*, **214**, 799 (1955).