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Review: The Role of Coenzymes in Clinical Enzymology

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ABSTRACT

Coenzymes participate in many of the enzyme analyses performed in the clinical laboratory. Supplementation of assay systems with optimal levels of coenzymes has recently been recommended as part of efforts to achieve interlaboratory standardization of enzyme measurements. Aspartate aminotransferase and alanine aminotransferase require pyridoxal phosphate for expression of enzyme activity. The role of this coenzyme in enzymatic transamination and the effects of its supplementation on the clinical estimation of these two enzymes is reviewed. Other coenzymes discussed are flavins, coenzymes for glutathione reductase, glucose oxidase, cholesterol oxidase and diaphorase, as well as thiamine pyrophosphate, coenzyme for transketolase. Catalase and peroxidase are used as examples of hemoproteins utilized in clinical measurements. Two peptide coenzymes, colipase and glutathione, are also considered. Measurement of apoenzyme stimulation upon supplementation with specific coenzymes is discussed as a valuable technique for quantitative coenzyme measurements or assessment of vitamin nutritional status.

Introduction

Enzymes have emerged as powerful diagnostic and analytical tools of the clinical laboratory. The number of enzymes of diagnostic interest continues to grow, as has the number of analyses performed using enzymes as reagents. In particular, reference methods[40] are being developed which utilize the high specificity offered by enzymatic analyses. Unfortunately, considerations of coenzyme requirements are often neglected. For example, although aspartate aminotransferase (AspAT),* a pyridoxal phosphate (pyridoxal-P)-dependent enzyme, has been recognized as diagnostically, important for 25 years,[23,29] the routine inclusion

* Nonstandard abbreviations and enzyme identification: pyridoxal-P, pyridoxal 5'-phosphate; AspAT, aspartate aminotransferase (L-aspartate; 2-oxoglutarate aminotransferase, EC 2.6.1.1; formerly known as glutamate oxalacetate transaminase, GOT); AlaAT, alanine aminotransferase (L-alanine; 2-oxoglutarate aminotransferase, EC 2.6.1.2; formerly known as glutamate pyruvate transaminase, GPT). Other enzymes are identified by their recommended name[26] and enzyme commission number when first cited in the text.

of pyridoxal-P into the assay has only recently been proposed.[4,25,44,46] Recommendations now call for enzyme analyses to be performed at optimal coenzyme concentrations.[6]

This review focuses upon coenzymes which are important in their relationship to enzymes measured or used in the clinical laboratory.

Definitions

While some enzymes depend solely upon their structure as proteins for activity, a great many also require nonprotein structures, or coenzymes. In general, coenzymes or cofactors are small, dialyzable, heat-stable components of the enzyme system. The intact enzyme-cofactor complex is termed a *holoenzyme.* When the coenzyme is removed from this complex, the remaining protein, called the *apoenzyme,* no longer possesses activity.

The distinction between coenzymes, cosubstrates and prosthetic groups is hazy. The term *prosthetic groups,* which is reserved for coenzymes that are very tightly bound, is a nebulous definition for it is difficult and illogical to fix an arbitrary limit—for example, a dissociation constant of 10^{-4}—to determine which coenzymes can be called prosthetic groups. Furthermore, the pyridine nucleotides (e.g., NAD^+) and ribonucleoside phosphates (e.g., ADP) are commonly called coenzymes, yet in nearly all clinical enzyme measurements they are truly cosubstrates, since they must be added to the reaction mixtures at concentrations near or equal to other substrates and are themselves converted into products during the course of the enzyme reaction. They are true coenzymes only *in vivo* where they are present at low concentrations and are being constantly regenerated by subsequent, supplementary enzyme reactions.

In this review, *coenzyme* means a compound which maintains its essential stoichiometry with the amount of enzyme in the system, rather than with the substrate or product.

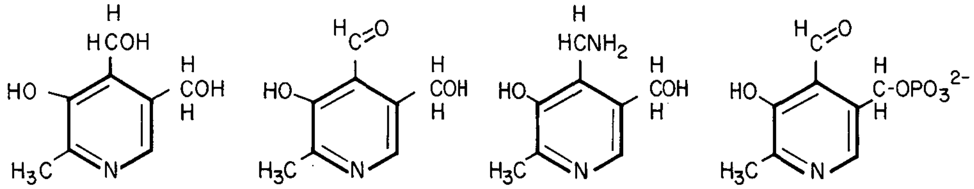
Vitamin B Coenzymes

Many coenzymes contain as active components certain trace substances, such as pyridoxine, riboflavin and thiamine, which are vital to the function of organisms and which are required as vitamins for certain species.

Vitamin B_6

One of the most versatile of the known coenzymes is pyridoxal-P: it is involved in transamination, dehydration, racemization, decarboxylation and dealdolization.[8,12,62] This is the biocatalytically active form of vitamin B_6 (figure 1).

Of the dozen or so enzymes which account for nearly all clinical enzyme mea-



I II III IV

FIGURE 1. Structures of vitamin B_6 compounds: I, Pyridoxol; II, Pyridoxal; III, Pyridoxamine; IV, Pyridoxal 5′-phosphate.

surements performed today, only the two transaminases—AspAT and alanine aminotransferase (AlaAT)—require a vitamin cofactor for enzyme activity. The participation of a coenzyme in aspartate-glutamate transamination was suggested by Kritzmann,[30] who in 1939 demonstrated that a heat-stable, dialyzable factor reactivated the dialyzed enzyme. In 1945 Green et al,[19] using partially purified preparations of AlaAT and AspAT, showed that pyridoxal-P participates in enzymatic transamination. The role of pyridoxal-P and pyridoxamine-P as cofactors for AspAT activity were subsequently confirmed by other workers. Snell,[61] using pyridoxal alone as a nonenzymatic model system, found that glutamate transaminated, with formation of pyridoxamine and 2-oxoglutarate. He postulated that the prosthetic group of AspAT is pyridoxal and that a similar transition occurs when this group binds to the enzyme. Meister et al,[37] using a partially purified porcine enzyme, also suggested such a shuttle mechanism. On the basis of many studies, pyridoxal-P is now firmly established as the cofactor of AspAT and other aminotransferases.

With the use of better purified AspAT and congruent nonenzyme model systems, there has been significant progress in describing the chemical features of the aminotransferase reaction.[7,8,62] The basic reaction involves the formation of a pyridoxal-P amino acid Schiff base (aldimine), which tautomerizes to a ketimine and subsequently hydrolyzes to the oxo acid and pyridoxamine-P. An abbreviated reaction scheme is presented in figure 2. There is evidence suggesting that when the enzyme is in the pyridoxal form, the carbonyl group forms an internal Schiff base with an ε-amino group from a lysyl residue near the active site. Thus a modification of this reaction consistent with the reaction catalyzed by AspAT or AlaAT would involve a transaldimination to intermediate II, rather than a dehydration. The internal aldimine between pyridoxal-P and lysine is thought to be responsible for the increased thermal stability of the enzyme in the pyridoxal form.[8,51] It has also been proposed[7,62] that interconversion of the reaction intermediates (II and III) shown in figure 2 is aided by a proximal enzyme amino group which serves as an acid-base catalyst; the ε-amino group of lysine, freed by transaldimination, may be involved.

In addition to the carbonyl-binding lysine residue, the existence of essential

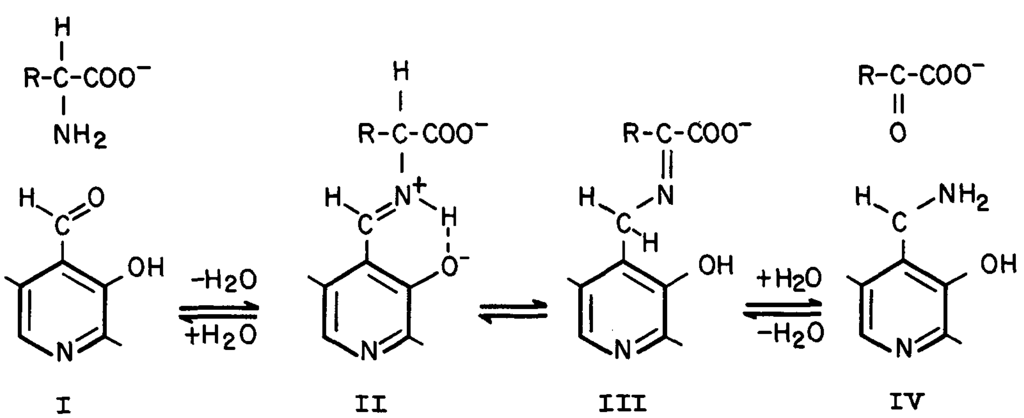


FIGURE 2. General mechanism for pyridoxal-catalyzed transamination; after Snell and others. I, amino acid and pyridoxal; II, aldimine intermediate; III, Ketimine intermediate; IV, oxo acid pyridoxamine.

arginine, cysteine, histidine and tyrosine residues has been suggested[7,18,52] The imidazole ring of histidine is also a candidate for catalyzing the aldimine-ketimine rearrangement.[7,15] Since the phosphate group of the coenzyme contributes greatly in binding it to the enzyme, most reports infer that there is a positively charged site on the protein and suggest that this is a functional arginine residue. However, there is some evidence indicating that the coenzyme is covalently linked through a threonine residue near the active center of the enzyme.[15]

Before 1972, pyridoxal-P, despite its well-recognized role, was seldom incorporated into any commonly used clinical assay for determining the AspAT or AlaAT activity of serum. Reports concerning its effect on serum AspAT activity differed. Karmen[29] was unable to show any measurable effect of pyridoxal-P or of boiled rat liver extract on serum aminotransferase activity. Similar conclusions have been reported by others.[38,56] Hamfelt,[20] however, was able to demonstrate increased serum AspAT activity after supplementation with pyridoxal-P. The increases he reported varied with the health and age of the subjects but were not limited to cases of vitamin B_6 deficiency.

In 1972, Rej et al[46] reported that supplementation by 25 μM pyridoxal-P effected an average increase of 16 percent in the apparent AspAT activity of 125 human sera by kinetic assay using phosphate buffer. The increase was not the result of increased enzymatic or nonenzymatic blanks but was partly attributable to the enzyme being protected against the loss of activity that occurs during preincubation with L-aspartate in phosphate buffer (figure 3). This stimulation of AspAT activity has been verified by other laboratories.[11,25,28,44,54,63]

Rej and Vanderlinde[49] further showed that the rate of association is markedly retarded by phosphate buffer, in contrast to Tris(hydroxymethyl)aminomethane

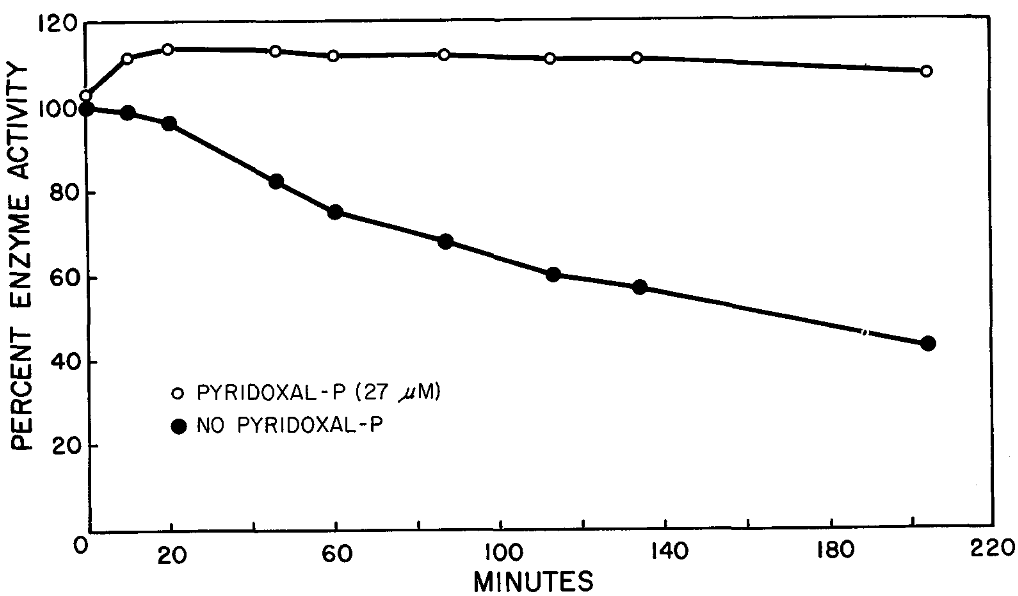


FIGURE 3. Effect of pyridoxal-P on the stability of serum-based human erythrocyte AspAT. AspAT was incubated with 134 mM L-aspartate and 89 mM phosphate buffer at 45°. At times indicated samples were withdrawn from the heating bath, cooled immediately and assayed for AspAT activity at 30°. Modified from reference 46, reprinted by permission.

(Tris) or six other buffers. Incubation with pyridoxal-P at 130 μmol per liter reactivated the entire apoenzyme portion of an apoenzyme/holoenzyme mixture within 5 min in Tris or Tricine buffers; in contrast, less than 20 percent was associated during 15 min in phosphate (figure 4). Fonda and Auerbach[17] confirmed that phosphate ion strongly inhibits the combination of coenzyme and apo-AspAT. Phosphate monoesters and sulfate also inhibited the rate of binding. Hørder et al[25] confirmed that measurement in Tris buffer results in rapid reassociation; they attempted to optimize conditions for complete apoenzyme saturation.

Rosalki and Bayoumi[54] suggested that serum apo-AspAT should be activated in the absence of L-aspartate, but other authors[25,39,49] have shown that full activation can be readily achieved during the usual incubation in the presence of L-aspartate.

While the magnitude of stimulation of AspAT activity by pyridoxal-P varied greatly among individual patient sera, the mean was consistently 30 percent for groups of sera with normal activities.[49] This is increased to 45 to 50 percent if "optimal" substrate concentrations (i.e., those proposed by the IFCC[4]) are utilized (figure 5).[24,25,39]

Jung et al[27,28] reported that the presence of pyridoxal-P alters the effect of temperature on the reactions of AlaAT and AspAT. The observed increase in the apparent Arrhenius slope may, however,

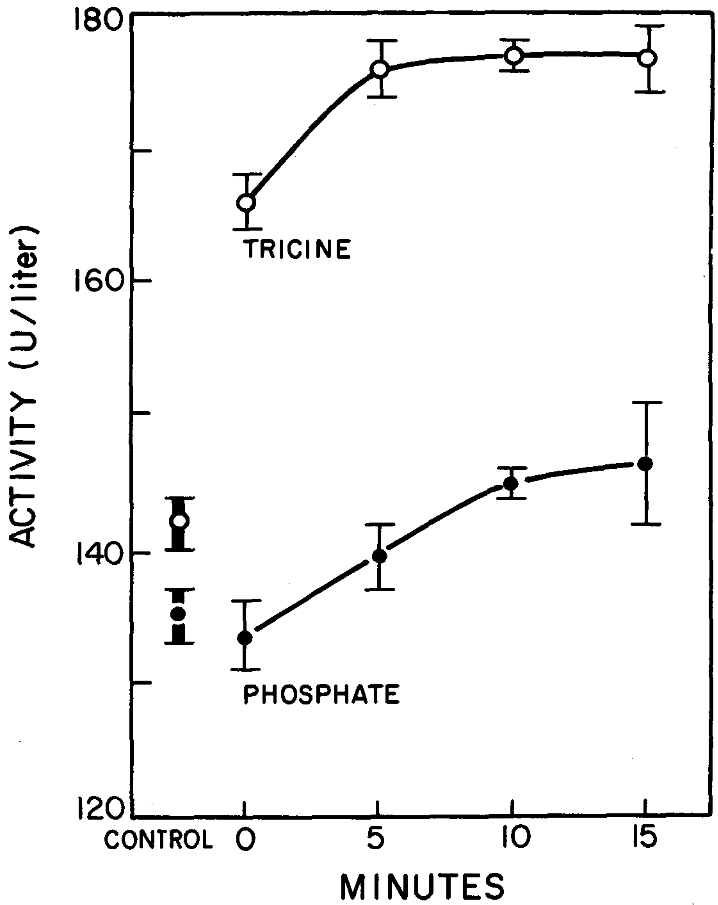


FIGURE 4. Effect of buffer on reassociation of an apo-AspAT/holoAspAT mixture with pyridoxal-P (130 μM). Control activities (broad error bars) were assayed without pyridoxal-P. Values shown are mean ± 1 SD. Results obtained with Tris buffer were virtually identical to those obtained with Tricine. L-Aspartate was present in the incubation mixture at 134 mM. Modified from reference 49, reprinted by permission.

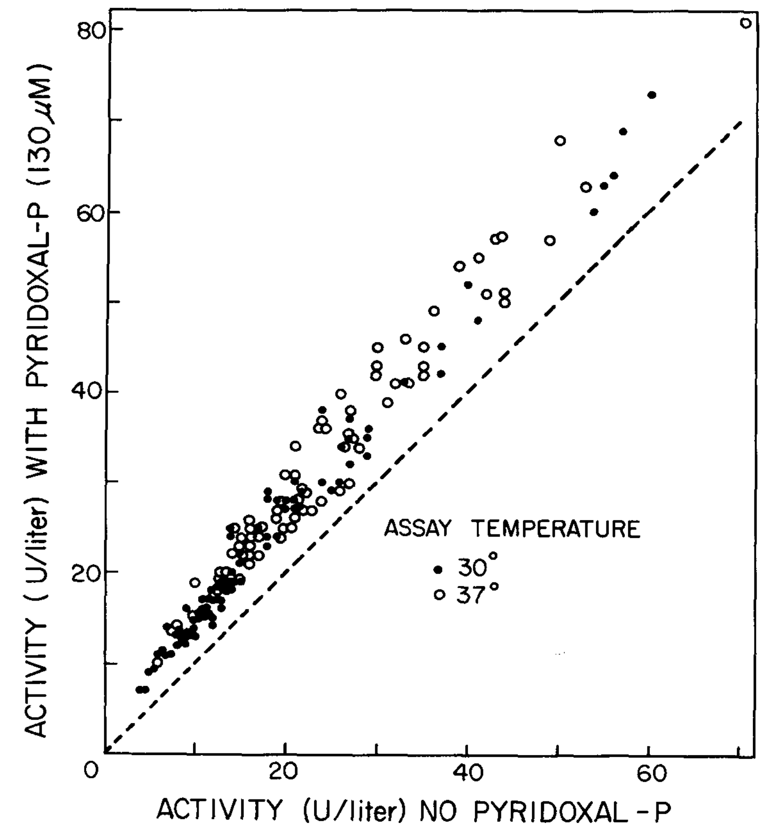


FIGURE 5. Effect of pyridoxal-P (130 μM) on the AspAT activity of patient serum at two temperatures. Average stimulation was 41 percent at 30° (N = 88) and 38 percent at 37° (N = 79). The average stimulation for sera containing AspAT activities within the normal range was 47 percent at 30° (N = 66) and 43 percent at 37° (N = 58). The broken line represents a slope of 1.0. Measurement conditions were 180 mM L-aspartate, 15 mM 2-oxoglutarate and 90 mM Tris buffer, pH 7.8.

be due merely to increased thermal stabilities of the enzymes in the presence of pyridoxal-P (figure 3), as these workers utilized phosphate buffer, which decreases the stability of AspAT.[49,51] Other researchers have shown that with Tris buffer the coenzyme, except for its stimulatory effect, does not influence the Arrhenius behavior of the enzyme in serum (figure 6).[45,50]

The addition of pyridoxal-P is analytically useful not only because of the increased enzyme activity (and concomitant change in absorbance) but also because it allows measurement of total AspAT activity in serum. When serum AspAT activity is measured without pyridoxal-P supplementation, the value determined is the sum of two variables; it is directly proportional to the total serum AspAT and inversely proportional to the pyridoxal-P saturation of the enzyme. With addition of exogenous pyridoxal-P, total AspAT can be measured independent of variables such as nutritional status, and the value is more representative of the total enzyme released in a disease state. Although the routine inclusion of pyridoxal-P in assays for AspAT will necessitate a redefined reference interval, such a change would be required of any new assay technique. Preliminary evidence with patient serum suggests that the interpretive value of the AspAT determination remains unchanged and that there is less scatter of values within the normal range.[49] Hørder and Bowers[24] concluded that fluctuations in serum AspAT activity of normal individuals are unaffected by supplementation with pyridoxal-P. Blank activity has been reported[25] to increase two- to eightfold

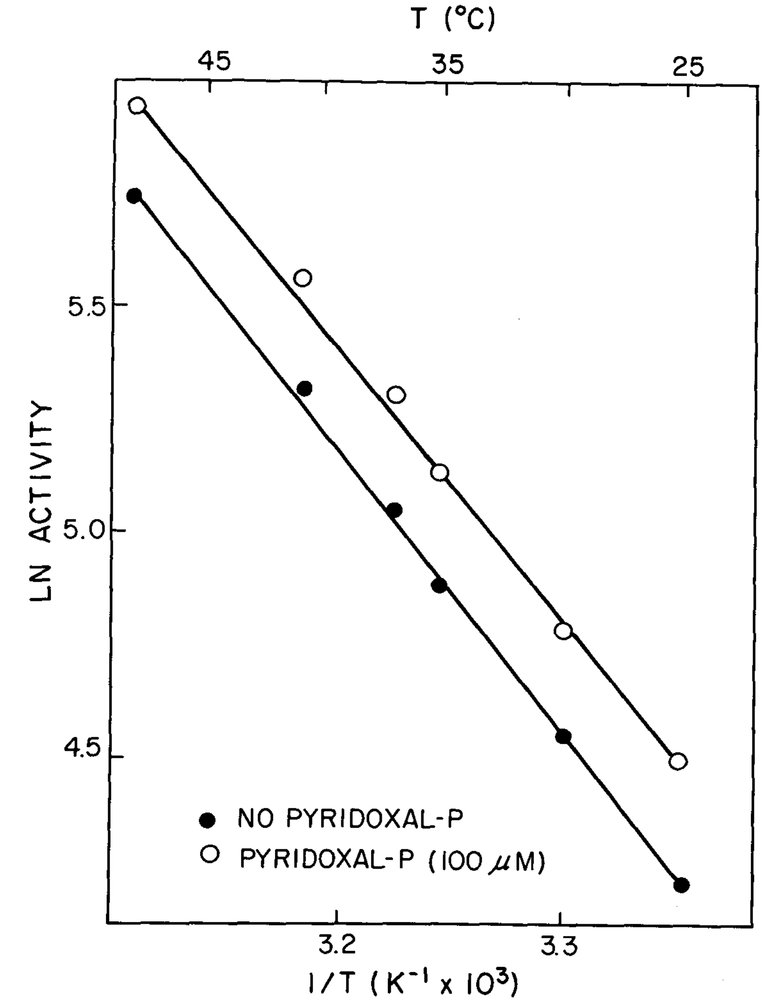


FIGURE 6. Effect of pyridoxal-P on the Arrhenius behavior of human serum AspAT. Conditions were 90 mM Tris buffer, pH 7.8, 180 mM L-aspartate and 15 mM 2-oxoglutarate. There was no significant difference between the slopes with or without pyridoxal-P.

upon supplementation of the AspAT assay mixture owing to the presence of apo-AspAT in the auxiliary enzymes, but this blank remains <1 U per liter if the proper reagents are used.[46,49]

An analogous situation exists for measurement of AlaAT activity. In this laboratory, addition of pyridoxal-P results in an average stimulation of 20 percent,* similar to that reported by others.[11,63] In contrast, Lustig[32] reported an increase of 170 percent for 22 normal sera preincubated with pyridoxal-P. However, in an investigation of reaction conditions and coenzyme stimulation, Jung and Egger[27a] recently confirmed the lower figure.

At high concentrations, pyridoxal-P binds to lysine residues of AspAT at other than the catalytic site.[34] It also interacts with lysine residues of other proteins including serum albumin.[35,49] Pyridoxal-P can therefore be used as an active-site probe for some enzymes, e.g., NAD(P)-oxidoreductases.[68] The transfer of pyridoxal-P from albumin to apo-AspAT has been described by Fukui and coworkers.[35] Rej and Vanderlinde[49] suggest that the addition of amino compounds

* Unpublished observation, R. Rej.

(Tris, L-aspartate) reverses the binding of the coenzyme to lysine residues through the formation of Schiff bases with these primary amines (figures 7 and 8).

It has been erroneously reported[59] that AspAT is inhibited by L-thyroxine and that this effect can be reversed by increased concentrations of pyridoxal-P. Although thyroxine does function so with other transaminases (e.g., tyrosine aminotransferase [EC 2.6.1.5]), it has no effect on the activity of human AspAT nor on the stimulation of activity by pyridoxal-P.[48]

There may also be some diagnostic importance in the degree of stimulation of transaminase activity observed in the presence of pyridoxal-P, perhaps relating to the length of time the enzyme is present in the peripheral circulation or to vitamin B_6 status. This stimulation can be quantitated by measuring erythrocyte or serum AspAT (or AlaAT) activity without and then in the presence of exogenous pyridoxal-P.[3,42,67] The degree of stimulation is related inversely to erythrocyte or serum vitamin concentration. Williams[67] and Bayoumi and Rosalki[3] recently described convenient routine protocols for the assessment of vitamin B_6 status utilizing this apoenzyme activation technique, and their results suggested good correlation between stimulation of AspAT activity and poor vitamin B_6 status.

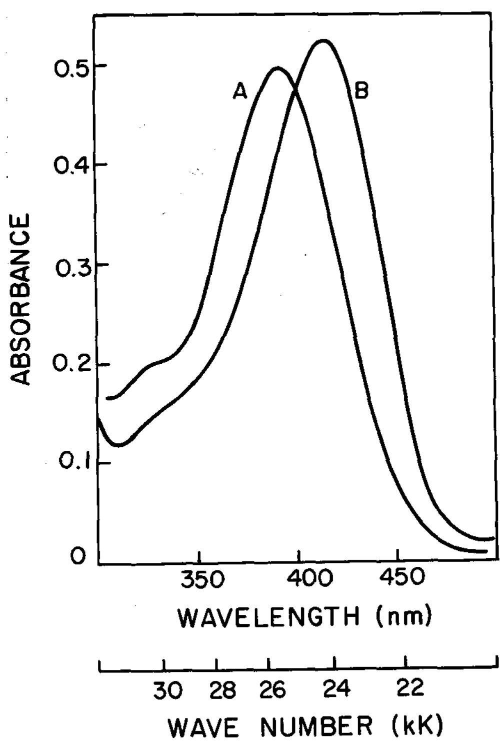


FIGURE 7. Absorbance spectrum of pyridoxal-P in 93 mM phosphate buffer, pH 7.5 (A) and in 93 mM Tris buffer, pH 7.5 (B). Wave number in kiloKaysers ($m^{-1} \times 10^5$). In the absence of amino compounds, the coenzyme shows an absorbance maximum at 390 nm, typical for the aldehyde form of pyridoxal compounds. In Tris buffer, the absorbance maximum is shifted to 415 nm, which is typical for spectra of pyridoxal Schiff bases. From reference 49, reprinted by permission.

Driskell et al[14] found that measurement of erythrocyte AlaAT activity with coenzyme stimulation is the most sensitive indicator of vitamin B_6 inadequacy. The degree of activation of erythrocyte AspAT by pyridoxal-P may also be directly related to the age of the erythrocytes.[16] Similar activation techniques for quantitative assay of pyridoxal-P[21,57] utilize the purified apoforms of AspAT and other pyridoxal-P dependent enzymes (e.g., tyrosine decarboxylase [EC 4.1.1.25]). These coenzyme stimulation methods have been used to monitor vitamin B_6 levels during pregnancy or in persons using oral contraceptives.[14,22]

Pyridoxal-P is also functional in assays which measure not enzymes but substrates. One example is histidine decarboxylase (EC 4.1.1.22), which is required in the manometric assay of histidine.

VITAMIN B_2

Riboflavin, or one of its derivatives, was detected as early as 1879 in the form of an impure pigment then called lactochrome.[33] Not until the early 1930's, however, was vitamin B_2 isolated and its importance as a cofactor in the yellow enzyme from yeast recognized. In 1935, its structure was confirmed as 6,7-

dimethyl-9-(1′-*D*-ribityl) isoalloxazine (figure 9).[66] Shortly after its discovery, the cofactor for the yellow enzyme was demonstrated not to be riboflavin but the 5′-phosphate-flavin mononucleotide (FMN).

The discovery of D-amino acid oxidase, a flavoprotein containing a cofactor distinct from FMN, led to the discovery of a second flavin cofactor—flavin adenine dinucleotide (FAD), in which the riboflavin monophosphate and adenoside monophosphate are linked by pyrophosphate. The majority of flavoproteins contain FAD rather than FMN (table I).

Some flavin enzymes can be separated into the apoenzyme and coenzyme components by acidic pH treatment and dialysis. (As a corollary, the active protein of the old yellow enzyme could be regenerated from the apoprotein and coenzyme). However, the bonds are often much tighter, and the flavin groups do not generally dissociate from the enzyme; the dissociation constant is usually of the order of 10^{-9}.[13] In some cases (e.g., succinic dehydrogenase and monoamine oxidase) there is a covalent bond between FAD and the enzyme protein, and the 8-position of the isoalloxazine ring is attached to either a histidyl or cysteinyl residue.[58]

Flavoproteins catalyze dehydrogenation. These electron transport agents oxidize substrates (glucose, for example, in the case of glucose oxidase) to produce the reduced form of the enzyme-bound cofactor, that is, protonation of the 1 and 10 positions of the isoalloxazine ring (figure 9) and production of the now oxidized substrate (in this example: gluconic acid). The flavin can then be reoxidized by other electron transport agents or by molecular oxygen itself to form peroxide. This reoxidation forms the basis of a significant number of clinically useful measurements (see peroxidases).

A number of flavin-containing enzymes are particularly interesting to the clinical laboratorian (table I). Decreased erythrocyte activity of glutathione reductase, an FAD-containing enzyme, is associated with congenital nonspherocytic hemolytic anemia, Gaucher's disease and α-thalassemia.[5,23] Vitamin B_2 deficiency results in an apparent decrease in glutathione reductase concentration, and addition of exogenous FAD to the assay mixture has been suggested[5,23] to eliminate this artifact. Even at normal enzyme concentrations, this supplementation increases glutathione reductase activity.[5]

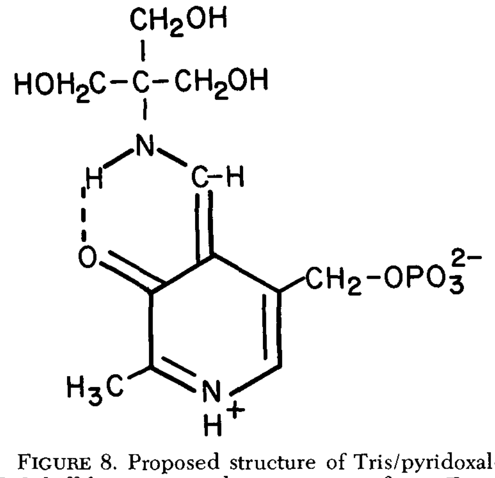


FIGURE 8. Proposed structure of Tris/pyridoxal-P Schiff base, quinoid or eneamine form. From reference 49, reprinted by permission.

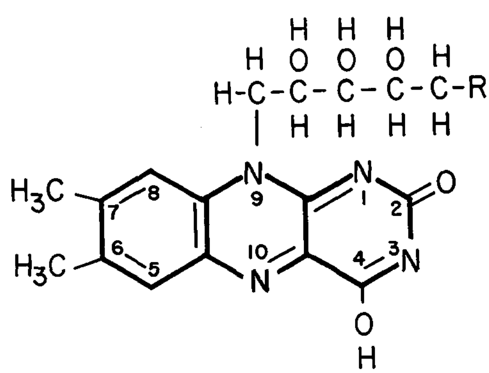


FIGURE 9. Structure of flavin cofactors (oxidized forms). R = OH, riboflavin; R = phosphate, flavin mononucleotide (FMN); R = adenosine diphosphate, flavin adenine dinucleotide (FAD).

TABLE I

Flavin-containing Enzymes[2,13,26,33,53,60]

Enzymes of clinical importance	Coenzyme
Glutathione reductase (1.6.4.2)	FAD
Succinate dehydrogenase (1.3.99.1)	FAD
Xanthine oxidase (1.2.3.2)	FAD
Enzymes used in substrate analysis	
Glucose oxidase (1.1.3.4)	FAD
Monoamine oxidase (1.4.3.4)	FAD
Luciferase	FMN
Diaphorases (1.6.4.3 and 1.6.99.1)	FAD & FMN*
Cholesterol oxidase (1.1.3.6)	FAD
L-Aminoacid oxidase (1.4.3.2)	FAD & FMN*
D-Aminoacid oxidase (1.4.3.3)	FAD
Xanthine oxidase (1.2.3.2)	FAD

*Species differences observed.

Vitamin B_2 nutritional status can be assessed in a manner similar to that suggested for B_6, by the measurement of erythrocyte glutathione reductase activity followed by measurement of this activity in the presence of exogenous cofactor—FAD.[3,67]

VITAMIN B_1

Thiamine pyrophosphate ("cocarboxylase" [figure 10]) is a coenzyme for three types of enzymatic reactions: oxidative decarboxylation, nonoxidative decarboxylation and formation of ketols. In the last function, it is the cofactor for transketolase EC 2.2.1.1), a clinically important enzyme with broad specificity which catalyzes the formation of sedoheptulose 5-phosphate in the pentose monophosphate shunt. The transketolase reaction is:

D-xylulose 5-phosphate + D-ribose 5-phosphate $\rightleftarrows$
sedoheptulose 7-phosphate + D-glyceraldehyde 3-phosphate

The cofactor operates at the enzyme's active center by binding substrate at the 2-carbon of the thiazolium ring, forming a rather stable intermediate and thus allowing the appropriate rearrangements catalyzed by the enzyme to occur.

Vitamin B_1 status can be assessed by examining the activation of erythrocyte transketolase after *in vitro* addition of thiamine pyrophosphate, according to the general technique described for vitamin B_6.[3,67]

Heme Coenzymes

Proteins containing coenzymes derived from the porphyrin nucleus, e.g. hemoglobin and chlorophyll, are biologically important. Porphyrins are derived from the parent tetrapyrrole porphin by substitutions at positions 1 and 8 with a variety of side chains, particularly methyl, ethyl, vinyl and propionyl groups. Porphyrins chelate with such metal ions as magnesium, copper, cobalt, silver and, of course, iron. The cation in such chelates lies in the center of the ring structure.[33]

Hence, the iron chelate of protoporphyrin IX is a coenzyme for proteins involved in the transfer of oxygen and for enzymes catalyzing a variety of oxidations. The cytochromes, a class of redox enzymes whose primary function is to transfer electrons from flavoproteins to the molecular oxygen or other acceptors,

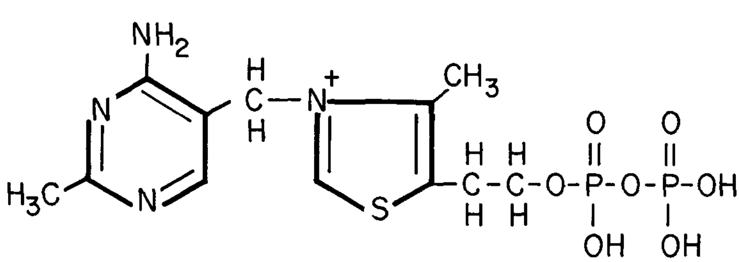


FIGURE 10. Structure of thiamine pyrophosphate.

employ heme as a firmly bound coenzyme. Heme also serves as a cofactor for catalase, which liberates molecular oxygen from hydrogen peroxide.[33]

Heme is particularly important for the analytical biochemist, since it serves at the catalytic site of the peroxidases (EC 1.11.1.7) which transfer electrons to peroxide oxidizing the hydrogen donor and producing water:

$$\text{Donor}_{\text{red}} + H_2O_2 \rightarrow \text{Donor}_{\text{ox}} + 2H_2O$$

The peroxidases show a broad specificity for hydrogen donors, including aniline, *p*-aminobenzoic acid, ABTS, ascorbate, NADH and *o*-dianisidine. The latter, when oxidized, forms a red-brown dye that absorbs at 420 to 480 nm.

Peroxidase is often used in conjunction with a specific oxidase (usually a FAD-containing enzyme) which generates hydrogen peroxide. For example, in the cholesterol determination of Röschlau et al,[55] cholesterol is hydrolyzed and then oxidized by cholesterol oxidase:

$$\text{Cholesterol} + O_2 \rightarrow \text{cholest-4-en-3-one} + H_2O_2$$

The hydrogen peroxide is used to oxidize methanol to formaldehyde by catalase (EC 1.11.1.6), itself a hemoprotein:

$$H_2O_2 + CH_3OH \rightarrow HCHO + 2H_2O$$

Formaldehyde is then quantitated using the Hantch condensation. This same chromogenic system can be used to measure uric acid (urate oxidase [uricase]; EC 1.7.3.3) or glucose (glucose oxidase).

Peptide Coenzymes

Colipase

Colipase, an effector necessary for pancreatic lipase (triacylglycerol lipase; EC 3.1.1.3) to be fully active upon triglyceride emulsions, has been purified from porcine pancreas. This cofactor, which is largely or entirely peptide, has molecular weight of about 9,000.[36] Although this small protein may be considered an enzyme subunit, it possesses the classical properties of a dialyzable, heat-stable coenzyme. Chapus et al[9,10] have reported that colipase is necessary for the adsorption of lipase to substrate micelles in the presence of bile salts. It is apparently essential for optimal lipolysis of long-chain triglycerides by pancreatic lipase, as removal of the cofactor diminishes enzyme activity.[9,10] Bile salts, which are often included in clinical assays of lipase activity,[65] are stimulatory over a very small range (ca 1 to 2 mM). They are inhibitory at higher concentrations, but this can be reversed by adding colipase.[43] Rathelot et al's[43] recommended optimal conditions for the determination of lipase assay include 6 mM bile salt in the presence of excess colipase.

Glutathione

The tripeptide coenzyme, γ-glutamylcysteinylglycine or glutathione, is a ubiquitous component of animal tissues and is present in erythrocytes at concentrations of 28 to 52 mg per dl of whole blood.[23] Glutathione is a substrate for two clinically important enzymes: glutathione reductase (see FAD-dependent enzymes) and γ-glutamyltransferase (EC 2.3.2.2). It is also a nonspecific activator of creatine kinase (CK, CPK; EC 2.7.3.2), replaceable by a number of other sulfhydryl compounds, including cysteine, N-acetylcysteine and mercaptoethanol.[64] The glutathione reductase reaction with oxidized glutathione (GSSG):

$$\text{GSSG} + \text{NADPH} + H^+ \rightleftarrows 2\ \text{GSH} + \text{NADP}^+$$

is a potential interference in the kinetic determinations of CK.[64]

Glutathione is also a coenzyme for glyoxylase (lactoyl-glutathione) lyase; EC 4.4.1.5), formaldehyde dehydrogenase (EC 1.2.1.1), maleylacetoacetate isomerase (EC 5.2.1.2), DDT-dehydrochlorinase (EC 4.5.1.1), and maleylpyruvate isomerase (EC 5.2.1.4).[23,26,33,41] Several of these enzyme reactions dependent upon glutathione are bases for its specific analysis.[31] For example, in the glyoxylase reaction glutathione is converted into a thiolester of the glyoxal substrate. Subsequent hydrolysis regenerates glutathione and the hydroxy acid product.[41]

Conclusion

Although the participation of coenzymes in enzymatic reactions has long been recognized, their role in clinical laboratory measurements has been less secure. The relatively recent introduction of pyridoxal-P supplementation in AlaAT and AspAT analyses aroused controversy regarding not only the manner of its inclusion but even whether or not such supplementation is necessary. The prime objection to including pyridoxal-P in transaminase assays is the need to reevaluate years of clinical experience obtained using analytical techniques without supplementation. This accumulation of suboptimal data might have been lessened if standardization[6] had been initiated earlier.

The author feels that there is significant merit to supplemented assays and agrees with Moss[39] that since it can be done easily, optimal concentrations of necessary cofactors should be used in assays of AlaAT and AspAT. The adoption of a system of reference and routine methods employing coenzyme supplementation may also bring about greater interlaboratory agreement than, for example, now exists in the case of AspAT measurements.[47]

The reliability of apoenzyme/holoenzyme ratios, measured with stimulated or unstimulated enzyme assays, as assessments of Vitamins B_1, B_2 and B_6 nutritional status, is now well documented.[3,14,16,67]

The number of analytes which are estimated with the use of coenzyme-requiring enzymes is still increasing. Awareness of the role of these coenzymes may lead to enzyme-coupled systems more efficient than some now in use. Current assay technology, which has been applied to the immobilization of enzymes, could also be applied to immobilize certain coenzymes, which could then be recycled in the course of coupled reactions. This development would conserve the cofactors, which must now be supplemented at cosubstrate concentrations, and thus make these assays more economical to perform.

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