Twenty tubes were incubated for two weeks at 37°C, 10 closed with bacteriological cotton and 10 with our AP discs. Evaporation was 35% less for tubes sealed with AP discs as compared with cotton plug. When tubes sealed with AP discs were sterilized for 2 h at 200°C, the paper surface of the discs turned only slightly yellow. The cotton plugs turned dark brown and distillation substances from the cotton adhered to the inside of the tubes.

When tubes of liquid medium, closed with the AP discs, were sterilized at 30 pounds pressure for 1 h, and even though the pressure was released suddenly, all the caps remained in place, while more than half of the cotton plugs in the control tubes came out. The metallic cover permits vapours to leave the tube and equalize the pressure in tube and autoclave. (When the pressure drops in the autoclave it drops to the same degree in the tube, and the cover stays in place.)

Opening and closing of the tube on the occasion of seeding or withdrawing colonies is also easier than with a cotton plug. Since the metal cap does not adhere perfectly to the walls of the tube, it does not give rise, when removed, to that phenomenon of an aspiration which may bring about air infection of the medium.

The external surface, the paper side, of the AP disc is white, and serves for any desired annotations with ink or otherwise. Such notes are much more reliable than those made on the tube, which may be effaced by repeated handling or by sterilization in the autoclave. Besides, the clean tube represents an economy of washing time.

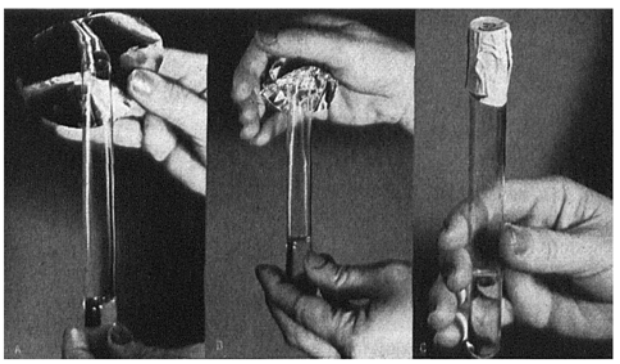
A single size of AP disc will fit a wide range of tube and flask sizes; they are much cheaper than cotton plugs, and require only $^1/_{100}$ the space for storage.

Discs of this material, aside from serving as caps, are very useful for holding material to be weighed on pharmaceutical or analytical scales, replacing the usual watch crystal in many cases.

Résumé. Un nouveau type de couvercle pour tube de microbiologie ou biochimie a été décrit. Il s'agit d'un disque d'aluminium doublé de papier. Il ferme plus étanchément que les bouchons, résiste à 200°C, conserve la stérilité, on peut écrire dessus et il est bon marché.

V. LORIAN[2]

Institute for Pathology of Tuberculosis, Cajú, Rio de Janeiro (Brazil), January 13, 1964.



A B C

A, The disc (metallic side) is applied to the mouth of the tube. B, it is pressed down with the palm of the hand. C, Ready.

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STUDIORUM PROGRESSUS

The Mechanism of Action of Glyceraldehyde-3-Phosphate Dehydrogenase

In our recent work on the hydrolytic reaction catalysed by GAPD[1], it was assumed that owing to its nucleophilic character a particular thiol group has an essential role[2]. It was established that the formation of an intermediate acetyl-enzyme during hydrolysis of an acyl-compound depends both on the electrophilic carbonyl carbon atom of the acyl-moiety of the substrate and on the structure of the leaving group originally bound to the acyl carbonyl carbon atom. The present paper is an attempt to study the nature of the influence which the structure of substrate and coenzyme has on the reaction of GAPD, and to analyse in detail the catalytic process.

In the reaction with aldehydes, such as GAP, GA, or acetaldehyde, the formation of the intermediate acyl-enzyme is accompanied by dehydrogenation and is followed by phosphorolysis[3-7] as shown below.

$$E\text{-}S^{\ominus} + NAD^{\oplus} + \underset{\underset{H}{|}}{\overset{\overset{O}{\|}}{C}}\text{-}R \rightleftharpoons E\text{-}S\text{-}\overset{\overset{O}{\|}}{C}\text{-}R + NADH \quad (1)$$

$$E\text{-}S\text{-}\overset{\overset{O}{\|}}{C}\text{-}R + HOPO_3^{2\ominus} \rightleftharpoons E\text{-}S^{\ominus} + \overset{\overset{O}{\|}}{R\text{-}C}\text{-}OPO_3^{2\ominus} + H^{\oplus} \quad (2)$$

[1] Abbreviations: GAPD = glyceraldehyde-3-phosphate dehydrogenase; GAP = glyceraldehyde-3-phosphate; GA = glyceraldehyde; *p*-NPA = *p*-nitrophenyl acetate; IAA = iodoacetic acid; NAD = nicotine amide-adenine dinucleotide; NADH = reduced nicotine amide-adenine dinucleotide; AMP = adenosine monophosphate; IMP = inosine monophosphate.

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Hypocatalysis. The reaction catalysed by GAPD is a rather complex one. In order to elucidate the mechanism of the action, dehydrogenation and acyl-enzyme formation should be studied separately. In the case of the reaction of aldehydes, however, the separation of these two elementary steps has not yet been achieved.

It was shown[8-10] that GAPD reacts not only with aldehydes, but also catalyses many other processes, like the ^{32}P-exchange of acetyl-phosphate and inorganic phosphate, the acetyl transfer from acetyl-phosphate to arsenate, or to thiol-compounds, the hydrolysis of *p*-NPA, etc. When compared with GAPD, the turnover numbers of the reactions mentioned are lower by approximately three orders of magnitude. All the catalytic processes are inhibited by IAA, suggesting that at least a part of the same active site participates in all catalytic processes. Study of the simpler processes, such as hydrolysis, has the advantage that it may help us to gain an insight into the mechanism of more complex reactions. It may lead to better understanding of the mechanism of a reaction catalysed *in vivo* to study a group of reactions – which might be called *hypocatalytic reactions* – catalysed by the same enzyme *in vitro*, and which will provide chemical evidence for the interpretation of the process occurring in nature.

By hypocatalysis we understand the enzymatic transformation of a non-specific substrate, carried out by a part of the active centre of the enzyme. This reaction does not necessarily include all the steps of the natural enzymatic process. The non-specific substrate should contain a functional group identical with that of the natural substrate. On the other hand, both substrates have to interact with the same amino acid residue of the enzyme.

According to the definition suggested, hydrolysis of *p*-NPA by chymotrypsin appears to be a hypocatalytic process. It has been shown that the same serine residue of the enzyme is involved in the hydrolysis of both *p*-NPA and the proteins[11]. From the chemical point of view there is an obvious relationship between peptide and ester bonds. In the case of GAPD, the same cysteinyl residue of the enzyme is required for the acyl-enzyme formation with both GAP and *p*-NPA[10]. The relationship between these two substrates is, at first sight, not quite obvious. The pre-requisite of acyl-enzyme formation is a substrate which contains both an electrophilic carbonyl carbon atom and a good leaving group attached to this carbon atom[2]. These structural requirements are inherent in *p*-NPA. In the case of GAP, however, they are only formed during the catalytic process. Consequently, the hydrolysis of *p*-NPA by GAPD may be regarded as a hypocatalytic process. The study of this phenomenon drew our attention to this potential property of the natural substrate, which may explain phospho-glyceryl-enzyme formation.

The Acyl-enzyme Formation. GAPD holds a position of particular interest among the dehydrogenases, as it firmly binds three NAD molecules (presumably consisting of three subunits), resulting in the formation of a yellow enzyme-coenzyme complex with an absorption maximum at approximately 360 mμ, as was shown by RACKER and KRIMSKY[9]. Compounds which react with thiol groups, such as IAA, *p*-chloromercury benzoate, H_2O_2 and iodine, cause this absorption band to disappear and, at the same time, inactivate the enzyme. The substrates, 1,3-diphosphoglycerate and acetyl-phosphate, also abolish this absorption band[12]. These data indicate an interaction between NAD and the thiol group which is involved in the formation of the acyl-enzyme, though the possibility of this phenomenon being the result of an unspecified conformational change due to the reaction of the thiol group cannot be excluded[4]. This latter possibility is improbable, however, taking into account that after treatment of the enzyme with various types of thiol reagents the absorption band at 360 mμ uniformly disappears. On the other hand, the different rates of IAA reaction in the presence and in the absence of NAD indicates a close proximity between the reacting thiol group and the coenzyme[12]. A further indication for the existence of such interaction is the formation of a ternary complex with silver ion, NAD and the mercaptide group of GAPD[13]. Accordingly, the assumption of an interaction between NAD and the thiol group of GAPD seems to be quite justified.

There are considerable discrepancies in opinions concerning the nature of the bond between GAPD and NAD. Covalent[3] as well as non-covalent[14] types of bonds have been suggested for the bond between the NAD pyridinium ring and the acyl-enzyme forming thiol group. The most probable way of interaction is a kind of charge-transfer as described by KOSOWER[15] who suggested the possibility of a charge-transfer interaction between the pyridinium ring of NAD and the sulfhydryl groups of the enzymes. In the case of GAPD this appears to be possible on the following grounds:

(1) The enzyme-NAD complex has, due to the multiplicity of the vibrational states associated with the weak bond between donor and acceptor, a fairly wide absorption band which is also characteristic of charge-transfer interaction.

(2) It was observed that donors, which form complexes with the pyridinium ring, become associated with the 4-position, while those which do not form such complexes, or do so only moderately, react at the 2-position of the pyridinium ring[15]. Dithionite, in contrast with other chemical agents and physical methods, reduces NAD to a NADH which is identical with the coenzyme reduced *in vivo*. During this reaction the existence of a charge-transfer complex between the sulphoxylate ion and the pyridinium ring can be demonstrated[16].

On the basis of the fairly rapid reaction between the charge-transfer forming thiol group and IAA, the former may be supposed to be a mercaptide ion. This mercaptide ion is presumably located at the pyridinium ring near the positively charged nitrogen atom, adding its electron to the upper molecular orbital of the π-electron system of the pyridinium ring. This interpretation of the charge-transfer complex may explain on stereochemical grounds the activation at the 4-position, namely the 2-position is probably masked by the large negative mercaptide ion located near the nitrogen atom.

Stereochemical conditions favour the orientation of the aldehyde group of the substrate with respect to this

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charge-transfer complex. It can be seen from Figure 1 that bonds might be formed between the carbonyl carbon atom and the mercaptide ion on the one hand, and between the aldehyde hydrogen and the 4-carbon atom of the pyridinium ring on the other hand. The positively charged pyridinium ring exerts an attraction on the hydrogen electron. Accordingly, the carbonyl carbon atom obtains a positive charge and can thus react with the nucleophilic mercaptide ion, forming an intermediate addition-compound. At this stage the electron-pair between the carbon and the hydrogen of the aldehyde is transferred to the position between the hydrogen and the 4-carbon atom of the pyridinium ring. In this state the hydrogen atom can be regarded as a good leaving group. In fact, a hydride transfer takes place while S-acyl-enzyme is formed. Hydride transfer is a well known phenomenon in organic chemistry[17], and is also assumed to occur in NAD–NADH transformation[18]. It should be emphasized that dehydrogenation and formation of the acyl-enzyme take place simultaneously and in close correlation with each other. Such an interpretation of the two processes is in agreement with the experimental data which support the assumption of a direct transfer of the hydrogen from the aldehyde group to the coenzyme[19], and the unsuccessful efforts to separate experimentally dehydrogenation from acyl-enzyme formation also support the mechanism discussed above. This mechanism holds in the case, too, if a group other than the mercaptide ion, adjacent to the pyridinium ring, serves as electron donor in the charge-transfer complex.

Phosphorolysis. The arsenolysis of 1,3-diphosphoglycerate[6], the phosphate exchange of acetyl-phosphate[8] and the reduction of 1,3-diphosphoglycerate by NADH[20] require NAD for an optimal reaction rate. These experimental observations indicate that NAD is required for the phosphorolytic step too. Recently, direct evidence has been obtained on the transfer of the acetyl group of acetyl-enzyme, formed during the reaction with *p*-NPA, to the phosphate ion in the presence of NAD[21]. Our data in the Table are consistent with this result. Moreover, these data prove that NADH is not able to promote the splitting of the acetyl-enzyme. This is also in agreement with the results of KOEPPE et al.[6] and of KRIMSKY and RACKER[22], according to which NADH, formed in the course of enzymatic reaction is replaced before phosphorolysis by NAD on the surface of the enzyme.

Participation of NAD in the phosphorolytic step is a rather unexpected phenomenon. So far the only explanation for this phenomenon has been the changes induced by NAD[12,23,24] in the conformation necessary for the catalytic activity. It may be expected, however, that a direct interaction will occur between the positively charged pyridinium ring and a suitable anion. It is known that IAA reacts with the thiol groups of GAPD much more rapidly in the presence than in the absence of NAD, while the reaction rate of iodoacetamide is slower and not influenced by NAD[12]. We may interpret this phenomenon as an example of intramolecular catalysis. The electrophilic carbon atom of the IAA is directed into close proximity to the nucleophilic sulphur atom as a result of the interaction between the negatively charged carboxylic group and the pyridinium ring of the charge-transfer complex. Another confirmation of the existence of an interaction between anions and the pyridinium ring is the high increase in the absorption of the complex of the 3-acetyl-pyridine analogue of NAD by GAPD in phosphate buffer[25]. On the basis of the above experimental results,

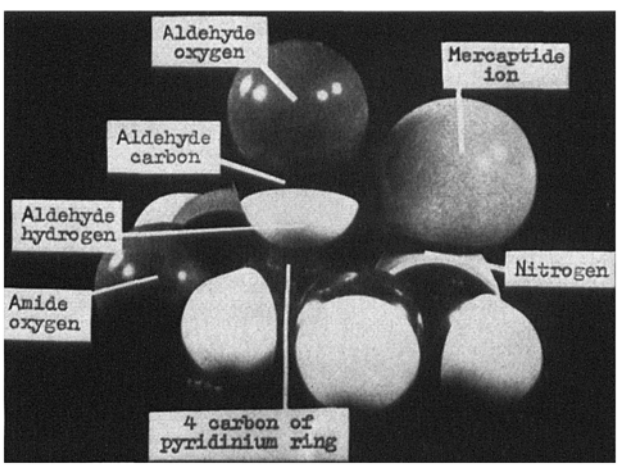

Fig. 1. A possible arrangement of the pyridinium ring of NAD, of the aldehyde group of the substrate and the mercaptide ion of GAPD at the beginning of the catalytic process (see text).

The effect of NAD and NADH on the phosphorolysis of acetyl-enzyme

Reaction mixture	No. of acetyl groups after 30 sec	Addition after 30 sec	No. of acetyl groups after 30 sec
GAPD+phosphate+*p*-NPA	1.60	none	1.56
GAPD+*p*-NPA	1.57	+NAD	1.53
GAPD+*p*-NPA		+NADH	1.53
GAPD+phosphate+*p*-NPA		+NAD	0.16
GAPD+phosphate+*p*-NPA		+NADH	1.39

The reaction mixture contained: 0.264 μM of NAD-free swine muscle GAPD; 2.78 μM of *p*-NPA; 3.0 μM of phosphate of NAD and NADH, respectively. The coenzymes were added after 30 sec, when the acetyl-enzyme had already been formed. The numbers represent the acetyl groups per mole of enzyme (M.w. 140,000) at the time indicated. The acetyl groups were determined by hydroxamic acid test after precipitation of the acetyl-enzyme and washing with trichloroacetic acid.

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it may be suggested that NAD can promote phosphorolysis not only by stabilizing the structure, but by its electrostatic attraction, too.

Helical structure of the Active Centre. Recent data have revealed the sequence around the thiol group which forms with the substrate the acyl-enzyme[26,27].

Lys-Ileu-Val-Ser-Asp(NH_2)-Ala-Ser-*Cys*-Thr-Thr-
1 2 3 4 5 6 7 8 9 10

Asp(NH_2)-Cys-Leu-Ala-Pro-Leu-Ala-Lys
11 12 13 14 15 16 17 18

This is an important basis on which to build a model of the active part of the enzyme. The low negativity of specific optical rotation of GAPD[23,24] indicates a high content of helices in the molecule. It was suggested that the part of the polypeptide chain of cytochrome c, which binds heam, contains two cysteine residues in an arrangement resembling the active site of GAPD, and has a helical structure[28]. We may assume, accordingly, that the part of the polypeptide chain which includes the two cysteine residues (Nos. 8 and 12) has a helical configuration. In this case the Asp(NH_2)-Cys (Nos. 11 and 12) doublet may be next to the the reactive cysteine No. 8. By such an arrangement of the two cysteine residues, it is possible to interpret the contribution of zinc and NAD to the active site.

Zinc-enzyme Complex. It was shown by VALLEE et al.[29] that GAPD contains zinc, and a possible role of the metal ion in the enzyme function was discussed by KELETI et al.[30,31]. Zinc is not removed by dialysis against water; moreover, in the native enzyme, there is no exchange between the bound zinc and ^{65}Zn[32]. These observations indicate a strong bond between the metal ion and the protein. There are only two amino acid residues which have a high affinity for zinc ion: the thiol group and the imidazole ring[33]. It is reasonable to suppose that this strong bond is a zinc-thiol bond. This is supported by the observations of FRIEDRICH et al.[34], who found that during photooxidation of GAPD three of the thiol groups are more protected than the others. This phenomenon might be due to the masking effect of zinc bound probably to these thiol groups. Furthermore, taking into account the effect of phenanthroline on the hydrolysis of *p*-NPA by GAPD, it is probable that it is cysteine No. 12 which binds the metal ion. It was found that phenanthroline, which readily forms a complex with zinc ion, inhibits the hydrolysis of *p*-NPA[2] which latter forms an intermediate acetyl-enzyme reacting with cysteine No. 8. The role of zinc in this catalytic process indicates that, to promote hydrolysis of the thiol ester, the metal should be situated in the proximity of the acyl-enzyme forming thiol group. Assuming the helical structure mentioned above, the zinc ion may indeed be located between the two cysteine residues.

The role of zinc in the hydrolysis of *p*-NPA by GAPD is in agreement with the observation of SCHWYZER and HÜRLIMANN[35] that the reactivity of thiol esters is strongly enhanced by metal ions which possess an affinity towards the sulphur atom. The attachment of zinc ion to the helix may further explain the increased nucleophilic character of the cysteine No. 8 residue. The existence of a mercaptide ion in a solution of pH 7 becomes understandable, because a positively charged ion would tend to repel protons and attract negatively charged ions. In this way a microenvironment with a pH higher than that of the bulk of the solution can develop around the zinc ion[36]. The possibility cannot be excluded, however, that the activation of the thiol group is brought about by an amino acid residue.

The Binding of NAD. The complex formation between the pyridinium ring of NAD and cysteine No. 8 was discussed above. This interaction is rather weak[37] and probably occurs only if a stronger enzyme-coenzyme bond has already been formed.

The pyrophosphate moiety of NAD was proposed for such a bond[38], with the possible participation of zinc[39]. An adenine-zinc interaction is, however, a more attractive proposition in view of the preference which zinc has towards nitrogen over oxygen as a ligand. The experimental data given below are in support of our assumption.

(1) Adenine has a tertiary imidazole-N and an amino group which can readily form a complex with zinc ion[40].

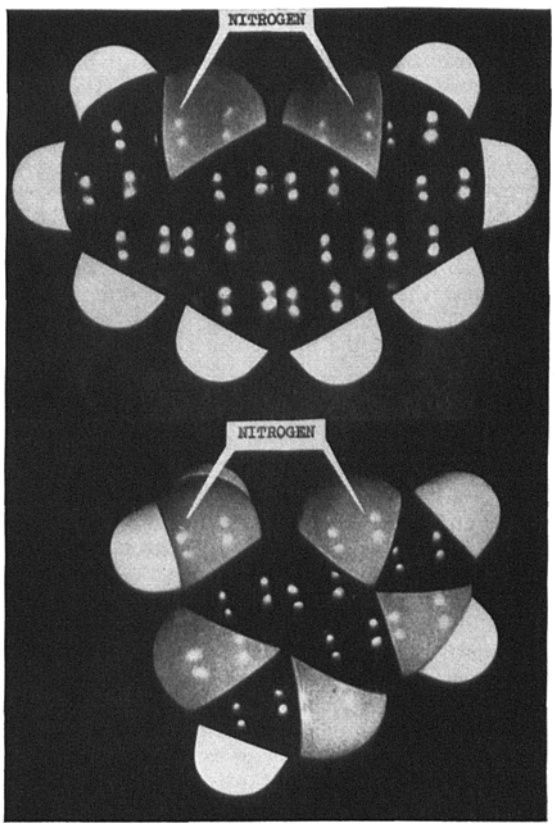

Fig. 2. Comparison of the arrangement of nitrogen atoms in adenine and phenantroline. Above: phenantroline; below: adenine (see text).

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(2) These two nitrogen atoms have a similar steric position as those of phenanthroline (Figure 2).

(3) In contrast to the free coenzyme, the bound NAD is not attacked by takadiastase deaminase[41].

(4) 3'-AMP and 5'-AMP have a much greater stabilizing effect on the NAD-free GAPD than 5'-IMP[42].

It follows from the decreased affinity of the 'oxidized' form of GAPD towards the pyridinium and the adenine moieties of NAD[41] that these two latter groups should be next to the cysteine No. 8 and thus next to each other. This assumption is supported by spectrofluorometric measurements of VELICK[43] concerning NADH binding which suggest a folded conformation for the bound coenzyme.

The formation of a zinc-adenine complex seems possible. The coordination number of zinc, usually 4 or 6, depends upon the ligand used. In the case of ethylene diamine, however, the first two bidentates are bound more readily than the third one[44]. Presumably the adenine moiety of the coenzyme represents this weak type of ligand in the protein-NAD-zinc complex (see below).

The Binding Site of Substrate. The specific acetylation of the amino group of a lysine residue has been demonstrated during the hydrolysis of *p*-NPA by GAPD[45]. Bromoacetic acid inhibits not only the hydrolytic reaction, but also N-acetyl-lysine formation. This observation suggests that the acetyl group is transferred from the acetyl-enzyme forming thiol group to the lysine residue in the proximity of the active centre. Moreover, it was demonstrated that the stable N-acetyl-enzyme exhibits a decreased activity towards GAP as substrate while the GA oxidation and *p*-NPA hydrolysis remain intact. Recently an octapeptide (Nos. 1 to 8) with two acetyl groups was isolated from the peptic hydrolysate of the acetyl-enzyme formed with *p*-NPA[27]. Although in the latter case it is not yet known which residue is responsible for the binding of the other acetyl group, it is probable that, due to the acetyl transfer occurring during the catalysis, this acetyl group is bound to the amino group of the lysine No. 1 residue.

The significant decrease of activity of N-acetylated GAPD towards GAP suggests the lysine residue as a part of the active centre which binds the phosphate moiety of GAP. This assumption is supported by our recent observation[42] that *p*-NPA hydrolysis by N-acetylated GAPD can be inhibited by a significantly higher concentration of GAP than the concentration required for inhibiting the native enzyme.

The Relation of the Active Site to the Whole of the Protein. It may appear from this paper that the helical peptide part discussed above is able to catalyse all processes. This helix may indeed be the most important part of the active site, which is required for all of the reactions mentioned. There should, however, be other parts of the polypeptide chain contributing other groups to form the complete active centre. There is no histidine residue in the helix, though its participation has been postulated in the catalysis[46]. On the basis of the effect of photooxidation on GAPD[34], it is not impossible that one imidazole is actually situated near the active centre. The enormous loosening of the structure, which occurs at the beginning of photooxidation, suggests that should such a histidine really exist, it will contribute first of all to the formation of the stable structure of the active centre. According to a very attractive hypothesis, another part of the polypeptide chain is bound to the zinc ion through this histidine. In this case the capacity of the zinc ion to form a bridge is similar to that of a disulphide bond. In fact, GAPD is denatured if the metal ion is removed by dithiols[31].

With respect to the relation of the active centre to the whole of the protein, the participation of bound NAD in the stabilization of the tertiary structure should first be considered. Optical rotation and proteolytic digestibility studies have demonstrated that NAD-free GAPD possesses a significantly looser structure than the enzyme with bound NAD[12,23,24]. Moreover, a rapid denaturation of GAPD can be observed even at 0°C, after removal of NAD with charcoal. As mentioned above, 3'-AMP and 5'-AMP protect effectively NAD-free enzyme at room temperature against spontaneous denaturation, while 5'-IMP is a weaker stabilizing agent[42]. In the light of the model of the active site, it seems to be probable that coenzyme binding influences the structure of the GAPD-metal complex, resulting in a change of protein conformation. In the NAD-free enzyme, the zinc ion is apparently bound through its $4s4p^3$ tetrahedral orbitals, which change for octahedral bonds once the coenzyme has joined the GAPD-metal complex. In Figure 3 a possible arrangement of the ligands around the zinc ion is shown. On the basis of model experiments[47] we attribute a role to the oxygen atom of the Asp(NH_2) (No. 11), since owing to its position it is capable of joining the zinc ion, together with the sulphur atom of cysteine No. 12, in the form of a bidentate. X_1 and X_2 are unknown ligands originating from a different part of the polypeptide chain; they too are illustrated as bidentate. One of them is probably the tertiary nitrogen atom of a histidine residue. If we regard the different sections of the polypeptide as components of the whole protein, the latter might be considered as a tetradentate, responsible for the strong enzyme-metal bond. The two nitrogens in the octahedron are parts of the adenine ring of the coenzyme. The acyl-enzyme forming thiol group is located above the polyhedron. Such an arrangement of the ligands offers an explanation for the inhibition of the acyl-enzyme hydrolysis by phenanthroline or by AMP, since these reagents penetrate between the zinc and the thiol ester.

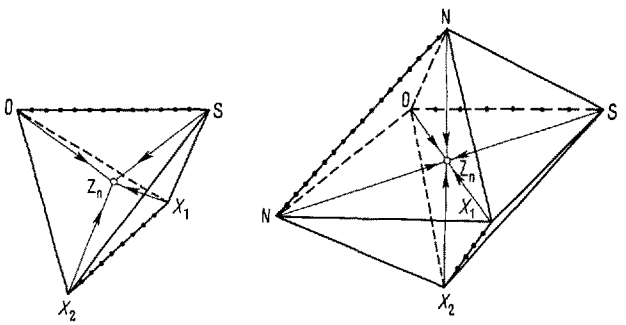

Fig. 3. Zinc complex formations in GAPD. On the left: tetrahedral form; on the right: octahedral form (see text).

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The proper conformation and the stabilization of the active centre is ensured by the forces which hold together the active parts in the polypeptide chain. Consequently, a small change in the tertiary structure may alter the position of the polypeptide sections, which participate in the formation of the active centre and might thus have a far-reaching influence on the enzymatic activity.

Zusammenfassung. Zur Erforschung des Wirkungsmechanismus einer komplexen Enzymreaktion vom Typus der GAPD können die mit der Wirkung dieses Enzyms verknüpften hypokatalytischen Vorgänge gewisse Anhaltspunkte geben. Als hypokatalytische Vorgänge werden die Reaktionen bezeichnet, die durch einen Teil des natürlichen aktiven Enzyms GAPD auf unspezifische Substrate ausgeübt werden. Auf Grund der Analyse einer solchen Reaktion, d. h. der Hydrolyse des *p*-NPA durch GAPD, wird die natürliche Funktion des Enzyms diskutiert.

Während der Hydrolyse des *p*-NPA wird die ε-Aminogruppe eines Lysinrestes der GAPD spezifisch acetyliert, die möglicherweise einen Teil des Bindungsortes der GAPD bildet.

Auf Grund unserer Experimente und mit Hinblick auf frühere Publikationen anderer Forscher wird ein mögliches Modell des aktiven Bereichs der GAPD vorgeschlagen.

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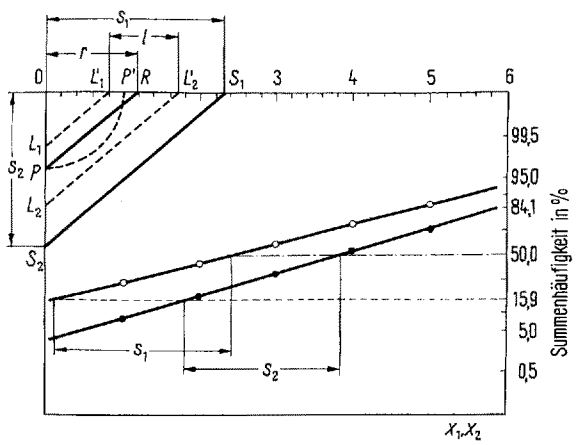
COGITATIONES

Graphisches Rechnen auf normalem Wahrscheinlichkeitspapier

Das bei der Auswertung von Versuchsergebnissen oft gebrauchte normale Wahrscheinlichkeitspapier findet vor allem Anwendung bei der (wenn auch nicht mathematisch exakten, so dennoch für die Praxis hinreichenden) Schnellprüfung auf die Normalverteilung und bei der Abschätzung des Mittelwertes und der Standardabweichung der Messwerte[1]. Zweck der vorliegenden Mitteilung ist es, zu zeigen, dass sich bei Vorliegen der Normalverteilung die vorgedruckte lineare Teilung des normalen Wahrscheinlichkeitsnetzes besonders gut dazu eignet, verschiedene graphisch-arithmetische Operationen, vor allem die graphische Multiplikation und Division, mit dem auf übliche Weise graphisch gefundenen Mittelwert bzw. der Standardabweichung vorzunehmen. Dadurch wird es möglich, gewisse Signifikanzprüfungen bzw. statistische Masszahlen, die auf demselben Mittelwert bzw. derselben Standardabweichung beruhen bzw. von diesen Werten als Grundmasszahlen abgeleitet werden können, direkt auf dem Wahrscheinlichkeitspapier auf eine anschauliche und bequeme Weise zu berechnen.

Als Beispiel möge vor allem die graphische Durchführung der Signifikanzprüfung auf den Unterschied zweier Standardabweichungen beschrieben werden. Diese besteht in ihrer Konfidenzintervallform nach DAVIES et al.[2] darin, zu untersuchen, ob sich der Wert 1 ausserhalb oder innerhalb des Konfidenzintervalls des Quotienten der zwei Standardabweichungen befindet. Die genannte Signifikanzprüfung ist zum Beispiel beim Vergleich der Reproduzierbarkeiten zweier Messverfahren von Bedeutung.

Falls ein normales Wahrscheinlichkeitsnetz vorliegt, in dem die Abszissenachse die lineare Teilung trägt, während die Summenhäufigkeitsprozente auf der Ordinatenachse aufgezeichnet sind[3], so empfiehlt es sich, die genannte Prüfung in der linken oberen Ecke des Netzes durchzuführen. Man lege am oberen Netzrand eine im Punkt *O* (siehe Figur) als dem Nullwert beginnende und nach rechts fortschreitende lineare Skala an mit numerierten Teilstrichen und einem Modul, der demjenigen gleich ist, der bei der Eintragung der Messwerte gebraucht wird. Diese wird im folgenden als die obere horizontale Skala bezeichnet. Analog denke man sich in der Richtung nach unten eine Zahlengerade mit dem Punkt *O* als Ursprung und mit dem gleichen Modul, die als linke senkrechte Skala bezeichnet wird. Auf ihr sehe man jedoch von der



Graphische Signifikanzprüfung über den Unterschied zweier Standardabweichungen. o—o Summenhäufigkeitsprozente für x_1; •—• Summenhäufigkeitsprozente für x_2. Weitere Erläuterung im Text.

[1] ERNA WEBER, *Grundriss der biologischen Statistik*, 4. Aufl. (VEB Gustav Fischer Verlag, Jena 1961), p. 109.

[2] O. L. DAVIES et al., *Statistical Methods in Research and Production*, 3. Aufl. (Oliver and Boyd, London and Edinburgh 1958), p. 58.

[3] Wahrscheinlichkeitspapiere «Schleicher & Schüll Nr. 298½» bzw. «Schäfers Feinpapier Nr. 500» u. a.