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The nature and mode of action of oxidation enzymes

Nobel Lecture, December 12, 1955

Practically all chemical reactions in living nature are started and directed in their course by enzymes. This being the case, Man has of course since time immemorial seen examples of what we now call enzymatic reactions, e.g. fermentation and decay. It would thus be possible to trace the history of enzymes back to the ancient Greeks, or still further for that matter. But it would be rather pointless, since to observe a phenomenon is not the same thing as to explain it. It is more correct to say that our knowledge of enzymes is essentially a product of twentieth-century research.

Enzymes are a sort of *catalyzers,* and in this connection a reminder of the origin of the concept catalysis may be in place. It was put forward by a school fellow of mine from Linköping High School. I never had the honour of meeting him, as he was 124 years older than I. It was one of the founders of Karolinska Institutet and the Swedish Medical Society, Jöns Jacob Berzelius, who in 1835. wrote in his yearbook:

"This is a new force producing chemical activity and belonging as well to inorganic as organic nature, a force which is undoubtedly more widespread than we have hitherto imagined, and whose nature is still concealed from us. When I call it a new force I do not thereby mean to say that it is a capacity independent of the electrochemical relations of matter, on the contrary, I cannot but presume it to be a particular manifestation of these, but as long as we cannot understand their reciprocal connections it will facilitate our researches to regard it for the time being as an independent force, just as it will facilitate our discussion thereof if it be given a name of its own. I shall therefore, to use a derivation well-known in chemistry, call it the *catalytic force* of bodies, decomposition through this force *catalysis,* just as with the term *analysis* we describe the separation of the constituent parts of bodies by means of ordinary chemical affinity. The catalytic force appears actually to consist therein that through their mere presence, and not through their affinity, bodies are able to arouse affinities which at this temperature are slumbering..."

Enzymes are the catalyzers of the biological world, and Berzelius' descrip-

tion of catalytic force is surprisingly far-sighted - one is tempted to say prophetic. Especially is one struck by his expressly refusing to believe that other than chemical forces are here in play; no, if one could once understand the mechanism it would doubtless prove that the forces of ordinary chemistry would suffice to explain also these as yet mysterious reactions.

Almost a hundred years were to pass before it became clear that Berzelius had been right. The year 1926 was a memorable one. The German chemist Richard Willstitter gave a lecture then in Deutsche Chemische Gesellschaft, in which he summarized the experiences gained in his attempts over many years to produce pure enzymes. Through various adsorption methods he had removed more and more of the impurities in some enzymes; especially had he worked with a so-called peroxidase, an enzyme of general occurrence in the vegetable kingdom. Finally, there was so little substance left that on ordinary analysis for e.g. protein, sugar, or iron, the solutions gave negative results. But the "catalytic" enzyme effect was still there. Willstätter drew the conclusion that the enzymes could contain neither protein, carbohydrate nor iron and that they did not belong to any known class of chemical substances at all, and he was inclined to believe that the effects of the enzymes derived from a new natural force, thus taking the view that 90 years earlier Berzelius had dismissed as improbable.

That same year, through an irony of fate, the American researcher J. B. Sumner published a work in which he claimed to have crystallized in pure form an enzyme, urease, from "jack-beans". It splits urea into carbon dioxide and ammonia. Sumner had got his crystals in rather considerable quantities with the help of much simpler methods than those applied by Willstätter in purification experiments on other enzymes. Sumner´s crystals consisted of colourless protein. In the ensuing years J. H. Northrop and his collaborators crystallized out a further three enzyme preparations, pepsin, trypsin, and chymotrypsin, like urease, hydrolytic enzymes that split linkages by introducing water.

If these discoveries had been undisputed from the outset it would probably not have been 20 years before Sumner, together with Northrop and Stanley, received a Nobel Prize. But it was not so easy to show that the beautiful protein crystals really were the enzymes themselves and not merely an inactive vehicle for the actual enzymes. Both Sumner and Northrop adduced many probable proofs that what they had produced really were pure enzymes, but no absolutely conclusive experiment could be brought forward, and as a matter of fact this was at that time probably not possible, for the

simple reason that their preparations appeared to consist of only colourless protein. At that time, and even today, for that matter, the methods of separation and analysis were scarcely sufficiently refined definitely to exclude the occurrence of small quantities of impurities in a protein preparation. From many quarters, accordingly, objections were raised to Sumner's and Northrop's results, and for obvious reasons especially the Willstatter school made itself heard in this connection.

The yellow enzyme

When in 1933 I went on a Rockefeller fellowship to Otto Warburg's institute in Berlin, Warburg and Christian had in the previous year produced a yellow-coloured preparation of an oxidation enzyme from yeast. It was obviously very impure in respect of the high-molecular constituent parts, which consisted chiefly of polysaccharides. The yellow colour was of particular interest: it faded away on reduction and returned on oxidation with e.g. oxygen, so that it was evident that the yellow pigment had to do with the actual enzymatic process of oxido-reduction. It was possible to free the yellow pigment from the high-molecular carrier substance, whose nature was still unknown, for example by treatment with acid methyl alcohol, whereupon the enzyme effect disappeared. Through simultaneous works by Warburg in Berlin, Kuhn in Heidelberg and Karrer in Zurich the constitution of the yellow pigment (lactoflavin, later riboflavin or vitamin B,) was determined.

It was here for the first time possible to localize the enzymatic effect to a definite atomic constellation: hydrogen freed from the substrate (hexose monophosphate) is, with the aid of a special enzyme system (TPN-Zwischenferment) whose nature was elucidated somewhat later, placed on the nitrogen atoms of the flavin (1) and (10), giving rise to the colourless leucoflavin. This is reoxidized by oxygen, hydrogen peroxide being formed, and may afterwards be reduced again, and so forth. This cyclic process then continues until the entire amount of substrate has been deprived of two hydrogen atoms and been transformed into phosphogluconic acid; and a corresponding amount of hydrogen peroxide has been formed. At the end of the process the yellow enzyme is still there in unchanged form, and has thus apparently, as Berzelius expressed himself, aroused a chemical affinity through its mere presence.

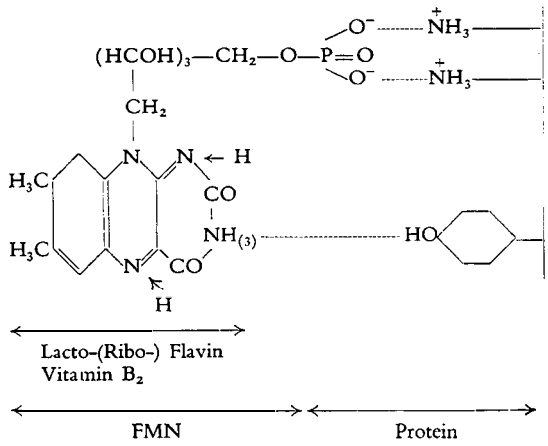

Fig.1.

But there now remained some extremely important questions to elucidate: why was the flavin in free form completely inactive, but active when it was anchored to a high-molecular carrier? And what was this carrier? In order to find this out I decided to try to purify the yellow enzyme, using for the purpose electrophoretic methods worked out by myself. At this time Tiselius had not yet worked out his technically more perfected electrophoretic methods, but my own proved surprisingly useful.

The polysaccharides, which constituted 80-90% of the entire weight, were completely removed, together with some inactive colourless proteins. After fractionated precipitations with ammonium sulphate I produced a crystalline preparation which on ultracentrifuging and electrophoresis appeared homogeneous. The enzyme was a protein with the molecular weight 75,000 and strongly yellow-coloured by the flavin part. The result of the flavin analysis was 1 mol flavin per 1 mol protein. With dialysis against diluted hydrochloric acid at low temperature the yellow pigment was separated from the protein, which then became colourless. In the enzyme test the flavin part and the protein separately were inactive, but if the flavin part and the protein were mixed at approximately neutral reaction the enzyme effect returned, and the original effect came back when one mixed them in the molecular proportions 1 : 1. That in this connection a combination between the pigment and the protein came about was obvious, moreover, for other reasons: the green-yellow colour of the flavin part changed to pure yellow, and its strong. yellow fluorescence disappeared with linking to the protein.

Developments in the flavin field were at this time (1934), to say the least,

hectic. For a while Richard Kuhn believed he was able to resynthesize the yellow ferment by bringing together lactoflavin and my colourless protein component; when his work was published, however, we were already aware in Berlin that his assumption was not correct. The point was that in my electrophoretic experiments lactoflavin behaved as a neutral body, while the pigment part separated from the yellow enzyme moved rapidly towards the anode and was thus an acid. An analysis for phosphorus showed 1 P per mol flavin, and when after a time (1934) I succeeded in isolating the natural pigment component this proved to be a lactoflavin phosphoric acid ester, thus a kind of nucleotide, and it was obvious that the phosphoric acid served to link the pigment part to the protein.

I will now show some simple experiments with the yellow enzyme, its coloured part, which we now generally refer to as FMN (flavin mononucleotide), and the colourless enzyme protein.

Exp. 1. A lantern-slide apparatus is furnished with two cuvette-holders and mirrors, so that a correctly presented image of the cuvettes may be projected on a white screen. A cuvette with a solution of yellow enzyme and another with FMN of the same molar concentration are shown simultaneously. The ferment-solution is pure yellow, the FMN-solution green-yellow, owing to the 1st that the light-absorption band in the blue of the free FMN is displaced somewhat in the long-wave direction on being linked with the protein component. A reducing agent ($Na_2S_2O_4$) is now added to the one cuvette, it is indifferent which. The colour disappears in consequence of the formation of leucoflavin. Oxygen-gas is bubbled through the solution: the colour comes back as soon as the excess of reducing agent has been consumed. The experiment demonstrates the reaction cycle of the yellow enzyme: reduction through hydrogen from the substrate side, reoxidation with oxygen-gas.

Exp. 2. A flask containing FMN-solution so diluted that its yellow colour is not descernible to the eye is placed on a lamp giving long-wave ultraviolet light. The solution gives a strong, yellow fluorescence which disappears on reduction and returns on bubbling with oxygen-gas.

Exp. 3. Two flasks are placed on the fluorescence lamp. The one contains a diluted solution of the free protein in phosphate buffer (pH 7), the other phosphate buffer alone. An equal amount of FMN-solution is dripped into each flask. In the flask with protein the fluorescence is at once extinguished, but in the flask with buffer-solution alone it remains. The experiment

demonstrates the resynthesis of yellow enzyme, and since the fluorescence is extinguished by the protein, one may draw the conclusion that some group in the protein is in this connection linked to the imino-group NH(3) of the flavin, which according to Kuhn must be free for the fluorescence to appear.

The significance of these investigations on the yellow enzyme may be summarized as follows.

1. The reversible splitting of the yellow enzyme to apo-enzyme + coenzyme in the simple molecular relation 1 : 1 proved that we had here to do with a pure enzyme; the experiments would have been incomprehensible if the enzyme itself had been only an impurity.
2. This enzyme was thus demonstrably a protein. In the sequel all the enzymes which have been isolated have proved to be proteins.
3. The first coenzyme, FMN, was isolated and found to be a vitamin phosphoric acid ester. This has since proved to be something occurring widely in nature: the vitamins nicotinic acid amide, thiamine and pyridoxine form in an analogous way nucleotide-like coenzymes, which like the nucleic acids themselves combine reversibly with proteins.

During the past 20 years a large number of flavoproteins with various enzyme effects have been produced. Instead of FMN many of them contain a dinucleotide, FAD, which consists of FMN + adenylic acid.

During the last few years technical advances have made it possible to attempt a chemical definition of the way in which the linkage between coenzyme and enzyme takes place. The now so-called "old" yellow ferment has in this connection once more proved to be an excellent object. We constructed a very sensitive apparatus to record changes in the intensity of the fluorescence, and were thus able to follow the rapidity with which the fluorescence diminishes when FMN and protein are combined, or increases when they are split. Under suitable conditions the speed of combination is very high. Thanks to the great sensitivity of the fluorescent method my Norwegian collaborator Agnar Nygaard and I were able to make accurate determinations of the speed-constant simply by working in extremely diluted solutions, where the speed of combination is low because an FMN-molecule so seldom happens to collide with a protein-molecule.

We now determined the reaction speeds under different conditions: we varied the degree of acidity, ionic milieu and temperature, and we treated the protein with a large number of different reagents which affect in a known way different groups in proteins. In this way we succeeded with a rather high

degree of certainty in ascertaining that phosphoric acid in FMN is linked to primary amino-groups in the protein, and the imino-group (3) in FMN to the phenolic hydroxyl group in a tyrosine residue, whereby the fluorescence is extinguished.

We still do not quite understand how through its linkage to the coenzyme the enzyme-protein "activates" the latter to a rapid absorption and giving off of hydrogen. But something we do know. The so-called oxido-reduction potential of the enzyme is in any case of great importance, and it is determined by a simple relation to the dissociation constants for the oxidized and for the reduced coenzyme-enzyme complex. The dissociation constants are in their turn functions of the velocity constants for the combination between coenzyme and enzyme and for the reverse process, and these velocity constants we have been able to determine both in the yellow ferment and in a number of enzyme systems. Without going into any details I may mention that the linkage of coenzyme to enzyme was found to have surprisingly big effects upon the potential of the former. In a number of cases the effect is strongly dependent upon variations in the salt content. Here we may suspect physiologically significant relations.

The alcohol dehydrogenases

I may now mention something concerning our work on the alcohol dehydrogenases, though I will here be relatively brief, as they are not well suited for experimental demonstrations in this lecture.

These enzyme systems, like the flavoproteins, consist of colourless protein in reversible combination with a coenzyme, which in the present case is the diphosphopyridinenucleotide, earlier generally referred to as cozymase, now "DPN", which has been elucidated in works by Hans von Euler, Karl Myrback, Ragnar Nilsson and others in Stockholm, and by Otto Warburg and Walter Christian in Berlin. It contains as active atomic group nicotinic acid amide, which analogously with the flavin in the yellow enzyme functions by taking up hydrogen from one direction and giving it off in another. It contains, further, ribose, phosphoric acid and adenine.

Alcohol dehydrogenases occur in both the animal and the vegetable kingdoms, e.g. in liver, in yeast, and in peas. They are colourless proteins which together with DPN may either oxidize alcohol to aldehyde, as occurs chiefly in the liver, or conversely reduce aldehyde to alcohol, as occurs in yeast.

The yeast enzyme was crystallized by Negelein & Wulf (1936) in Warburg's institute, the liver enzyme (from horse liver) by Bonnichsen & Wassén at our institute in Stockholm in 1948.

These two enzymes have come to play a certain general rôle in biochemistry on account of the fact that it has been possible to investigate their kinetics more accurately than is the case with other enzyme systems. The liver enzyme especially, we have on repeated occasions studied with particular

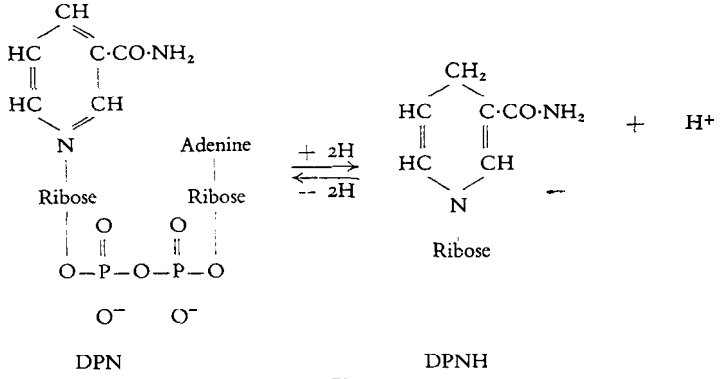

Fig. 2.

thoroughness, since especially favourable experimental conditions here presented themselves. For all reactions with DPN-system it is possible to follow the reaction $DPN^+ + 2H \rightleftharpoons DPNH + H^+$ spectrophotometrically, since DPNH has an absorption-band in the more long-wave ultraviolet region, at 340 mμ, and thousands of such experiments have been performed all over the world. A couple of years ago, moreover, we began to apply our fluorescence method, which is based on the fact that DPNH but not DPN fluoresces, even if considerably more weakly than the flavins. Asregards the liver enzyme there is a further effect, which proved extremely useful for certain spectrophotometrical determinations of reaction speeds; together with Bonnichsen I found in 1950 that the 340 mμ band of the reduced coenzyme was displaced, on combination with liver alcohol dehydrogenase, to 325 mμ, and together with Britton Chance we were thus able with the help of his extremely refined rapid spectrophotometric methods to determine the velocity constant for this very rapid reaction.

I shall not go into further details, but simply point out that extremely complicated reactions result from the fact that we are here dealing with a three-body problem containing the enzyme protein, the coenzyme and the

substrate, where, furthermore, both the coenzyme and the substrate occur in both oxidized and reduced forms.

A more or less complete system may be written thus that only after g steps does the enzyme become free to begin a new cycle; the net result is that the alcohol has given two of its hydrogen atoms to the coenzyme. Even this simplified schema means that one must determine 18 rate constants, two for each part-reaction, which is of course a formidable task. We have succeeded, however, in determining some of them as regards the yeast enzyme.

The kinetics of the liver enzyme is quite other than that of the yeast enzyme. Here we have to do with a simpler reaction process which can be expressed with only three equations and six velocity constants.

We have here been able to determine all the six constants at different degrees of acidity and with different salt concentrations, so that the reaction velocities of this enzyme system are probably at present the best known of all (Theorell, Bonnichsen, Chance, Nygaard).

The differences between the yeast and liver enzymes indicated here, explain why the yeast enzyme produces alcohol from aldehyde, while the liver enzyme does the contrary.

It is a curious whim of nature that the same coenzyme which in the yeast makes alcohol by attaching hydrogen to aldehyde also occurs in the liver to remove from alcohol the same hydrogen, so that the alcohol becomes aldehyde again, which is then oxidized further.

When we had studied the kinetics of the alcohol dehydrogenases it was a simple matter to use these to determine alcohol quantitatively, e.g. in blood samples. This so-called "ADH"-method is about as accurate as Widmark's method, only more sensitive and above all practically specific for ethyl alcohol. It is now legally introduced in forensic chemical practice in Sweden and in West Germany.

Hemin proteids

Even before going to Berlin in 1933 I had become interested in a close relative of hemoglobin, the myochrome or, as it was afterwards called, the myoglobin discovered spectroscopically by K. A. H. Mörner in 1897, and had crystallized it in 1932.

After my return home I set about purifying another hemin proteid, viz. cytochrome *c,* one of the "histohematins" or "myohematins" observed by

the Irishman Mac Munn in his home-made spectroscope at the end of the 1880's. Mac Munn's "hematins", after a period of obscure existence in petit type in larger textbooks, had been brought out into the light again by David Keilin in Cambridge in 1925. In 1936 we had obtained the cytochrome approximately 80% pure, and in 1939 close to 100%.

It is a beautiful red, iron-porphyrin-containing protein which functions as a link in the chain of the cell-respiration enzymes, the iron atom now taking up and now giving off an electron, and the iron thus alternating valency between the 3-valent ferri and the 2-valent ferro stages. It is a very pleasant substance to work with, not merely because it is lovely to look at, but also because it is uncommonly stable and durable. From 100 kg heart-meat of horse one can produce 3-4 grams of pure cytochrome *c*. The molecule weighs about 12,000 and contains one mol iron porphyrin per-mol.

Exp. 4. Two cuvettes each contain a solution of ferricytochrome *c*. The colour is blood-red. To the one are added some grains of sodium hydrosulphite: the colour is changed to violet-red (ferrocytochrome). Oxygen is now bubbled through the ferrocytochrome-solution: no visible change occurs. The ferrocytochrome can thus not be oxidized by oxygen. A small amount of cytochrome oxidase is now added: the ferricytochrome colour returns.

From this experiment we can draw the conclusion that reduced cytochrome *c* cannot react with molecular oxygen. In a chain of oxidation enzymes it will thus not be able to be next to the oxygen. The incapacity of cytochrome to react with oxygen was a striking fact that required an explanation. Another peculiarity was the extremely firm linkage between the red hemin pigment and the protein part; in contradistinction to the majority of other hemin proteids, the pigment cannot be split off by the addition of acetone acidified with hydrochloric acid. Further, there was a displacement of the light-absorption bands which indicated that the two unsaturated vinyl groups occurring in ordinary protohemin were saturated in the hematin of the cytochrome. In 1938 we succeeded in showing that the porphyrin part of the cytochrome was linked to the protein by means of two sulphur bridges from cysteine residues in the protein of the porphyrin in such a way that the vinyl groups were saturated and were converted to a-thioether groups. The firmness of thelinkage and the displacement of the spectral bands were herewith explained. This was the first time that it had been possible to show the

nature of chemical linkages between a "prosthetic" group (in this case iron porphyrin) and the protein part in an enzyme. Karl-Gustav Paul has since found an elegant method whereby to split the sulphur bridges with silver salts, and he has with organic chemical methods conclusively confirmed the constitution in this respect.

The light-absorption bands of the cytochrome showed that it is a so-called hemochromogen, which means that two as a rule nitrogen-containing groups are linked to the iron, in addition to the four pyrrol-nitrogen atoms in the porphyrin. From magnetic measurements that I made at Linus Pauling's institute in Pasadena and from amino-acid analyses, titration curves and spectrophotometry together with Å. Åkeson it emerged (1941) that the nitrogen-containing, hemochromogen-forming groups in cytochrome *c* were histidine residues, or to be more specific, their imidazole groups.

Recently we have got a bit farther. Tuppy & Bodo in Vienna began last year with Sanger's method to elucidate the amino-acid sequence in the hemin-containing peptide fragment that one obtains with the proteolytic breaking down of cytochrome *c,* and succeeded in determining the sequence of the amino acids nearest the hemin. The experiments were continued and supplemented by Tuppy, Paléus & Ehrenberg at our institute in Stockholm with the following result:

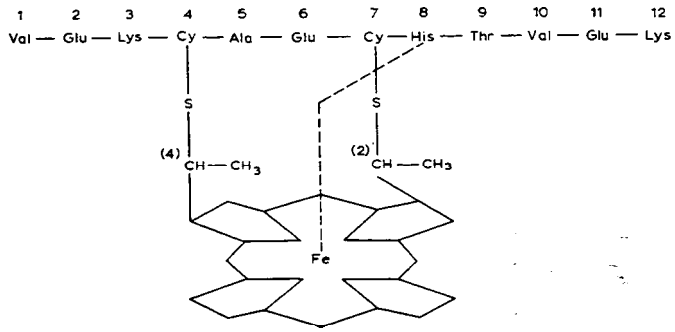

Fig. 3.

The peptide chain 1-12 ("Val") = the amino acid valine, "Glu" = glutamine, "Lys" = lysine, and so forth) is by means of two cystein-S-bridges and a linkage histidine-Fe linked to the hemin.

When in 1954 Linus Pauling delivered his Nobel Lecture in Stockholm he showed a new kind of models for the study of the steric configuration of

peptide chains, which as we know may form helices or "pleated sheets" of various kinds. It struck me then that it would be extremely interesting to study the question as to which of these possibilities might be compatible with the sulphur bridges to the hemin part and with the linkage of nitrogen-containing groups to the iron. Pauling was kind enough to make me a present of his peptide-model pieces, which I shall show presently. This is thus the second time they figure in a Nobel Lecture.

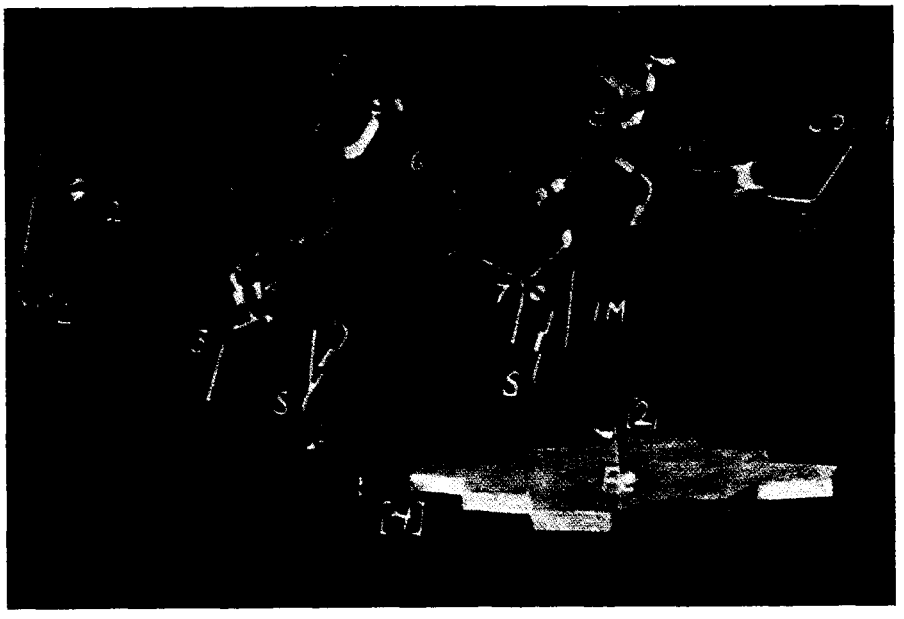

Fig. 4. Steric model of the hemopeptide remaining after pepsin-digestion of cytochrome c, constructed of metal parts representing the atomic distances and valency-directions. In the figure the peptide chain is disposed by means of hydrogen bonds as a left-twisting α-helix, seen at right angles to the longitudinal axis. Both the sulphur bridges (S) and the imidazole group (IM) of the histidine then fit their correct linkage-positions, and the peptide chain becomes parallel with the hemin plate *(the light, polygonal metal plate at the bottom of the picture). Acta Chem. Scand.*, 9 (1955) 1193.

Anders Ehrenberg and I now made a hemin model on the same scale as the peptide pieces and constructed models of hemin peptides with every conceivable variant of hydrogen bonding. It proved that many variants could be definitely excluded on steric grounds, and others were improbable for other reasons. Of the original, at least 20 alternatives, finally only one

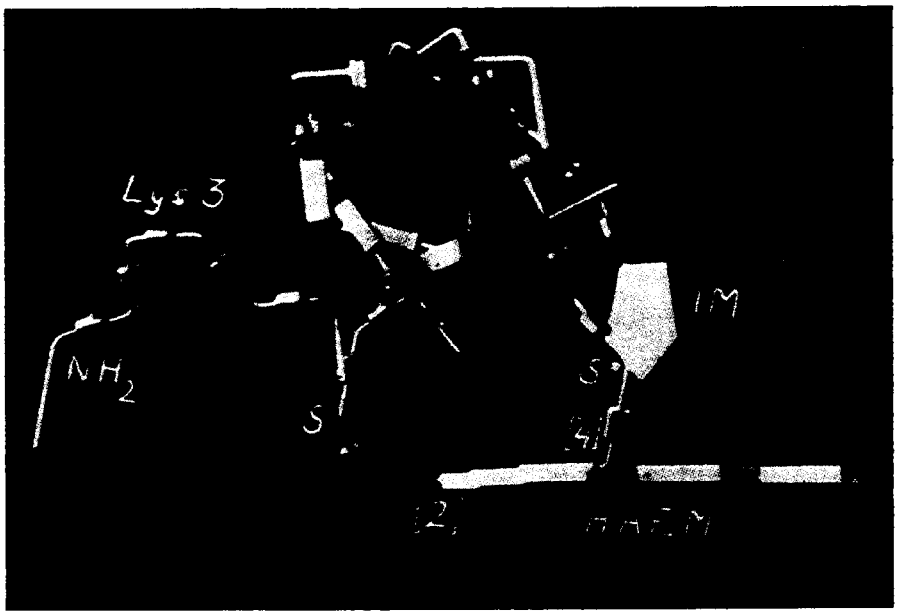

Fig. 5. The model seen in the longitudinal direction of the α-spiral. For the sake of survey-ability the side-chains have been fitted only to the amino acids Lys (3), Cy (4), Cy (7), and His (8). *Acta Chem. Scand.*, 9 (1955) 1193.

remained - a left-twisting a-helix with the cysteine residue no. 4 linked to the porphyrin side-chain in 4-position, and cysteine no. 7 to the side-chain in 2-position. The imidazole residue fitted exactly to linkage with the iron atom. The peptide spiral becomes parallel with the plane of the hemin disc.

I think it may be said that it was of considerable interest to have Pauling's and Corey's most important spiral confirmed with purely chemical methods, which in our case of course was possible thanks to the unique circumstance that we had a short peptide linked at no fewer than three places to a rigid structure, the hemin. After we had sent this work to the printer's there arrived from Arndt & Riley in England an X-ray-crystallographic confirmation that cytochrome *c* contains left-twisting α-helices.

Through calculations on the basis of the known partial specific volume of the cytochrome we now consider it extremely probable that the hemin plate in cytochrome *c* is surrounded by peptide spirals on all sides in such a way that the hemin iron is entirely screened off from contact with oxygen; here is the explanation of our experiment in which we were unable to oxidize reduced cytochrome *c* with oxygen-gas. The oxygen simply cannot get at

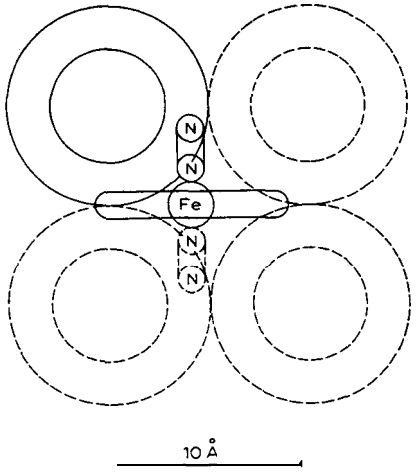

Fig. 6. Hypothetical section through a cytochrome-*c* molecule. *(Whole-drawn lines):* the hemopeptide. *(Dashed lines)*: parts of the natural molecule split off with pepsin. The region between the outer and inner circles is taken up by the lateral chains of the amino acids. The four peptide chains surround the iron atom (Fe), making it inaccessible to oxygen. *Acta. Chem. Scand.,* 9 (1955) 1193.

the iron atom. There is, on the other hand, a possibility for electrons to pass in and out in the iron atom via the imidazole groups.

One sixth of the entire steric structure of the cytochrome molecule is herewith elucidated, and we glimpse further possibilities of gradually elucidating the rest. It strikes us as interesting that even at this stage the special mode of reacting of the cytochrome is beginning to be understood from what we know of its chemical constitution.

Peroxidases and catalases

These, according to what we now know, are hemin proteids, thus close relatives of hemoglobin and cytochromes. Their effects were observed as early as in the 19th century, as regards the catalases already at the beginning, as regards the peroxidases in the middle. It was almost inevitable that the first to produce hydrogen peroxide, the Frenchman Thenard, should also discover the catalase effect; as soon as hydrogen peroxide is brought into contact with the majority of native biological materials the hydrogen peroxide is broken down with development of gas to oxygen + water. That the

catalases contain hemin as active group was made probable by Zeile & Hell-Ström at Hans von Euler's institute in 1930, and the first catalase, from cow's liver, was crystallized by Sumner & Dounce in 1937.

Exp. 5. To 3% H_2O_2 in phosphate buffer pH 7 is added 0.2 mg catalase. A vehement formation of gas is observed. When the bubbling has abated the contents are poured out and fresh H_2O_2 + buffer is added. The bubbling continues in consequence of minimal amounts of remanent catalase.

The peroxidases are of general occurrence in the vegetable kingdom. When in 1941 we crystallized a peroxidase for the first time, from horse-radish, it proved, contrary to all Willstätter's assumptions, to contain protein as well as carbohydrate and hemin, thus iron. The reason why Willstatter was unable to show the presence of any of these in his diluted solutions, which were nonetheless still active, was simply that Willstätter had happened upon a quite unusually strongly active enzyme. One thousandth of a milligram in one liter of solution still gives a distinct effect. The peroxidases do not break down hydrogen peroxide directly, but "activate" it to oxidize quite a number of hydroquinones, anilines or even nitrite.

Exp. 6. To H_2O_2 in acetate buffer, pH 4.6, is added a trace of peroxidase: no effect. Benzidine is now added: blue colouring.

Peroxidases occur here and there in animal material, e.g. leucocytes (verdoperoxidase, isolated in pure form by Kjell Agner 1941) and in milk (lactoperoxidase, isolated by myself and Åkeson a year or so later).

In the living cells both catalases and peroxidases function in the same way by using the hydrogen peroxide arising in connection with the reaction of oxygen with a number of autoxidable substances, such as Slavins, ascorbic acid, etc., to oxidize otherwise difficultly combustible substrates. Especially interesting is Agner's observation that the verdoperoxidase in the leucocytes + H_2O_2 can detoxify for example tetanus and diphteria toxins, which would of course explain one of the main functions of the leucocytes.

Catalases and peroxidases both give, with H_2O_2, first greenish complex compounds and then reddish ones. The study of these has presented immensely intricate problems, where we have had to use combinations of spectrophotometric and now very refined magnetic methods to try to elucidate the mechanism; Our American colleague Britton Chance, who in 1946-1948

worked at the Nobel Institute, has successfully carried investigations still further in this field.

To conclude: what is the final goal of enzyme research? The first stage is to investigate the entire steric constitution of all enzymes - a nice little job! So far we know the most easily accessible sixth part of the smallest enzyme molecule, cytochrome *c*.

In the second stage it is a matter of deciding how the enzymes are arranged in the cell-structures. This implies, as a matter of fact, the filling of the yawning gulf between biochemistry and morphology.