The N-terminal Amino Acid Sequence of Sheep Heart Myoglobin

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The N-terminal amino acid sequence of residues 1—50 of sheep heart myoglobin was determined using the automatic Edman degradation procedure. The amino acid phenyl-thiodyanthoins were identified by thin layer chromatography and/or by mass spectrometry.

A partial amino acid sequence of sheep heart myoglobin has been published by K. HAN et al. 1. The N-terminal sequence of residues 1—16 has not yet been determined since the N-terminal peptide appeared to be part of an insoluble core formed during tryptic digestion. In this case it seemed promising to apply the automatic Edman degradation procedure 2 to establish the amino acid sequence of the N-terminal portion of this protein. Sperm whale myoglobin 3 and horse myoglobin from skeletal muscles were used as control proteins under the assumption that the amino acid sequence of the latter protein is identical with that of horse heart myoglobin which has been already investigated 4.

Materials

Sheep heart myoglobin was isolated from heart muscles of local, randomly bred sheep according to the method of BÜNNIG and HAMM 5. Horse myoglobin from skeletal muscles was purchased from Serva, Heidelberg, Germany, and sperm whale myoglobin from Pierce Chem. Comp., Rockford, Ill., USA. The samples were further purified by chromatography on Sephadex G-100 using 0.05 M Tris/HCl buffer pH 7.95. The haem was removed by the method published by TEALE 6. The aqueous phase was extensively dialyzed against water at 4 °C and then lyophilized.

Methods

Edman degradation procedure: The automatic equipment for the stepwise Edman degradation procedure was built under the guidance of Mr. S. STRAUB by the workshop of the "Max-Planck-Institut für Virusforschung, Tübingen", according to the outlines given by EDMAN and BEGG 2 with the following modifications: a) The vacuum system consists of two 2-stages rotary gas ballast pumps, one for restricted vacuum and one for full vacuum. With this system a pressure of $1 \times 10^{-2}$ Torr can be obtained at the end of the vacuum steps after the extraction with ethylacetate and after the second extraction with 1-chlorobutane. The ultimate vacuum attainable in the system is approximately $3 \times 10^{-3}$ Torr. b) The bell jar is connected directly with the nitrogen pressure line, operated by a separate valve and no longer via 3-way valve. The oxygen content of the nitrogen was diminished by absorption using an Oxisorb column (Messer-Griesheim, Düsseldorf, Germany) to give a residual oxygen content of 1 ppm. c) The programming unit is a simple set of 26 electromagnetic timers.

Reagents and solvents were purified as described by EDMAN and BEGG 2 but employing an additional redistillation step throughout. All purified samples were kept separately in sealed glass ampules under nitrogen at $-15$ °C except for the solvents used for the extraction steps. The purity of trifluoroacetic acid, n-heptafluorobutyric acid and $N,N,N',N'$-tetrakis-(2-hydroxypropyl)-ethylenediamine was controlled by NMR- and infrared spectrometry.

Thinlayer chromatography was performed on silica gel (E, Merck, Darmstadt) using the following solvent systems: (a) chloroform/2-chlorethanol 100 : 2 (v/v), (b) chloroform/aceticacid/methanol 100 : 20 : 3 for those amino acid PTHs which migrate too slow in system (a). All solvents were freshly distilled except 2-chloroethanol which was treated first with solid tris-(hydroxymethyl)-aminomethane over night, filtered and distilled at 12 mmHg.

Mass spectrometry was performed with the LKB 9000 combination gaschromatograph/mass spectrometer. In most cases the use of the gaschromatograph could be omitted. The mass spectrometer was operated with an electron energy of 70 eV, with an ion source temperature of 250 °C and with an accelerating voltage of 3.5 KV. The mass spectra of the amino acid PTHs were evaluated according to the outlines given by HAGENMAIER et al. 7.

Results

The automatic equipment used for the Edman degradation studies corresponds to the equipment
described in detail by Edman and Begg. Some minor modifications are outlined in the methods section. The general operational procedure was the same as published by the above mentioned authors. It was found to be advantageous to dissolve the samples in 50% aqueous trifluoroacetic acid to give a protein concentration of 2 \( \mu \text{moles/ml} \). Before transferring the sample 0.15 ml ethanol were layered on the walls of the spinning cup. The protein solution containing 0.5 – 0.7 \( \mu \text{moles} \) of protein was placed as an overlay on the ethanol film. After replacing the bell jar the solvent was evaporated and the protein film extracted once with 1-chlorobutane followed by another evaporation step before starting the first degradation cycle. No delocalization of the protein film could be observed within 50 cycles.

The amino acid PTHs were identified by thin layer chromatography and/or by mass spectrometry. The N-terminal amino acid sequence of the sperm whale myoglobin sample could be followed by thin layer chromatography up to residue 30 and by mass spectrometry to residue 44 and was found to be identical with the sequence published by Edmundson. The N-terminal amino acid residues 1 – 34 of horse myoglobin from skeletal muscles could be identified by thin layer chromatography. Residues 14, 16, 26, 27, 30, 31 were also identified by mass spectrometry. Residues 35 – 50 could only be identified by mass spectrometry. The resulting amino acid sequence is identical with that of horse heart myoglobin.

The N-terminal amino acid of sheep heart myoglobin was found to be glycine and not valine as reported by Han et al. The residues 1 – 44 could be identified by thin layer chromatography and residues 45 – 50 by mass spectrometry. Control identifications of residues 13, 24, 31, 37 and 43 by mass spectrometry were also performed. The amino acid sequence of residues 17 – 50 was identical with that published by Han et al.

The N-terminal amino acid sequences of sheep heart myoglobin and horse myoglobin from skeletal muscles are given in Table 1.

**Discussion**

The automatic Edman degradation procedure has been found to be a reliable tool for sequence analysis. The repetitive yield in a single degradation cycle is comparable to that reported by Edman and Begg as judged by the purity of the amino acid PTHs of the higher cycle numbers. The loss of protein in each degradation cycle, however, must have been essentially higher than 2 percent. All attempts to reduce the loss of protein, i.e. by restriction of extraction steps, led to a reduction of the repetitive degradation yield and/or to an increase of impurities. At the present time it can not be predicted how many degradation cycles are feasible when mass spectrometry is used for the identification of amino acid PTHs. In the work reported the automatic degradation procedure was stopped after 50 cycles.

The finding of glycine as the N-terminal amino acid of sheep heart myoglobin instead of valine as reported by Han et al. can be due to the possibility that both groups deal with genetically different material. The N-terminal amino acid sequence of sheep myoglobin shows three amino acid exchanges within the residues 1 – 50 when compared with horse myoglobin and one exchange when compared with beef myoglobin.

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Table 1. Comparison of the N-terminal sequences of myoglobins from sheep heart and horse skeletal muscles.
The authors want to thank Mr. S. Straub and his coworkers for their excellent technical work and Dr. G. Nicholson for performing the mass spectrometric analysis.


Quantitative Structure-Activity Studies of Hydrazones, Uncouplers of Oxidative Phosphorylation

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α-Acyl-α-cyanocarbonyl-phenylhydrazones are effective uncouplers of oxidative phosphorylation. The \( p_{10}^{50} \)-values of a series of 60 hydrazones with substituent variations in six positions of the molecule were determined in rat liver mitochondria. They ranged from 4.96 to 7.06. The biological data together with physico-chemical compound and substituent parameters were analysed by multiple regression techniques to establish the structure activity relationship.

The integral parameters \( pK_a \) and \( \log P \) (partition coefficient) gave correlations of only moderate significance. Good agreement of found and calculated \( p_{10}^{50} \)-values was obtained by an equation with electronic (\( \sigma \)) and hydrophobic (\( \pi \)) substituent parameters in linear and quadratic terms. It is concluded that the contribution of a substituent to uncoupling activity depends on its position in the molecule. The activity is enhanced by hydrophobic shielding of the acidic NH-group. The relevancy of these results in relation to current theories on the mechanism of oxidative phosphorylation is discussed.

Reagents dissipating the link between formation of ATP and electron transport in mitochondria, the so-called uncouplers of oxidative phosphorylation, belong to different chemical classes. Recent compilations of uncouplers\(^1\),\(^18\) comprise such diverse compounds as dicoumarol, lauric acid, aromatic hydrazones, benzimidazoles, salicylanilides, methylamine and atebulin, apart from the classical nitrophenols. Furthermore, 1,1,3-tricyano-2-aminol-1-propene\(^2\), 3-nitropropionate\(^3\), 2-anilinothiophenes\(^4\), and 3′-tri-fluoromethyl-N-phenylthranilic acid\(^5\) are reported to be uncouplers. However, a critical comparison of the activity of these chemicals, expressed as the concentration necessary either for half-maximal stimulation of electron-transport in absence of ADP and Pi, 50%-inhibition of ATP-synthesis or half-maximal stimulation of ATP-ase, reveals that all those compounds with a high degree of activity have an acidic OH- or NH-group in common.

This is true for the classical uncouplers, 2,4-dinitrophenol as for other phenols, for salicylanilides, dicyanocarbonyl-hydrazones, benzotriazoles, benzimidazoles, trihalomimidazoles and phenylaminodinitrothiophenes. In contrast, carboxylic acids like lauric acid and 3-nitro-propionic acid or amines like methylamine and chloropromazine are comparatively weak uncouplers. Discussions on the mechanism of uncoupling action are usually based on the assumption that all acidic uncouplers act by the same general mechanism. For the OH-