ACTA CHEMICA SCANDINAVICA 17 (1963) 8–12

Studies on the Binding of Ferriheme to Poly-L-lysine

G. BLAUER and A. EHRENBERG

Weizmann Institute of Science, Rehovot, Israel, and Biochemical Department, Nobel Medical Institute, Stockholm, Sweden

Measurements of paramagnetic susceptibility on a red and a green complex of ferriheme with poly-L-lysine in aqueous solution, indicate that low-spin complexes are formed. For the red complex it is assumed that two ε-amino groups are bound to each porphyrin iron. Molecular models suggest that these two ε-amino groups originate from different polypeptide molecules. With these results the previously established spectral analogies between ferricytochrome *c* and the red polylysine complex are now extended to include similarities in the postulated mode of binding of the heme in both cases.

Recently new types of complexes between ferriheme (ferriprotoporphyrin IX) and synthetic polypeptides have been described [1]. The spectra of some of these complexes are similar to those of hemoproteins. Thus, a red compound which resembles ferricytochrome *c* spectrophotometrically was obtained by combining poly-L-lysine with ferriheme at pH 11, while at lower pH a sharp transition to a green compound was observed. It was concluded that specific steric effects are important factors in determining the type of macromolecular complex formed as poly-DL-lysine gave only a green complex at pH 11 and since low molecular weight poly-L-lysine did not form the red compound. Poly-L-lysine of not too low molecular weight has been shown to form an α-helix at high pH [2], which unfolded at lower pH. Therefore, it seemed reasonable to correlate the formation of the red complex with the interaction of ferriheme with the helical polypeptide, while in the green complex the polypeptide was considered to be unfolded. It now seemed of interest to ascertain the bond type involved in both complexes by magnetic susceptibility measurements and to determine their most probable structure by the use of molecular models.

PARAMAGNETIC SUSCEPTIBILITY

The apparatus used has been described earlier [3,4]. The complexes were prepared from alkaline solutions of ferriheme chloride. The total concentration of iron was 1.8×10^{-4} M and the poly-L-lysine was 7×10^{-3} monomolar (Mol. wt. ~5000). At higher concentrations of the complex, the solution is not homogeneous and precipitation occurs. Measurements were carried out at

20°C. Both the sample tube and the pipettes used were flushed before filling with CO_2-free air in order to avoid serious drifts of pH in the unbuffered solutions. Spectra and pH of the solutions measured were checked either before or after the magnetic measurement. Blank solutions consisted of all components except the ferriheme. From the mean difference in volume susceptibility between the complex and the blank solutions the molar value for the complex was calculated. For comparison, measurements were also made on solutions of alkaline ferriheme and of myoglobin in buffer of pH 6.5. The experimental data are collected in Table 1.

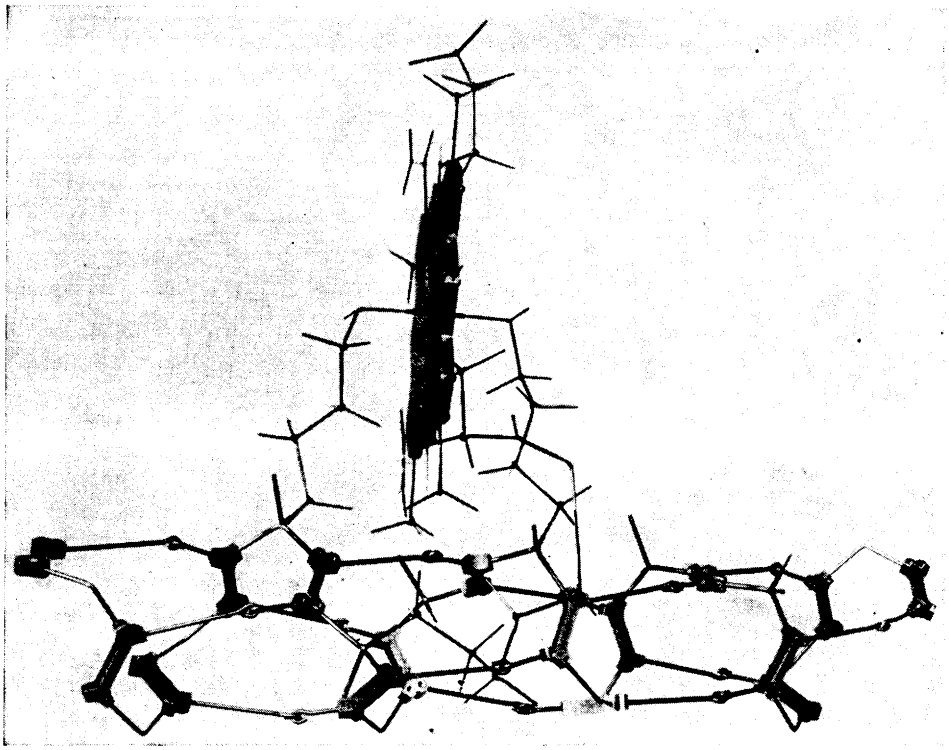
Table 1. Paramagnetic data of the two ferriheme-poly-L-lysine complexes and of the substances measured for comparison. The errors given for the susceptibilities are estimated maximal deviations.

Substance	$\chi_{Fe} \times 10^6$ cgs emu	μ_{eff} Bohr magnetons
Red complex of ferriheme and poly-L-lysine at pH 11	2 100 (± 400)	2.2
Green complex of ferriheme and poly-L-lysine at pH 8	3 100 (± 300)	2.7
Alkaline ferriheme (6×10^{-4} M)	13 500 (± 100)	5.7
Ferrimyoglobin (2.0×10^{-4} M) in 0.1 M phosphate at pH 6.5	15 000 (± 1000)	5.9

The paramagnetic susceptibility of the red complex is very close to that of ferricytochrome *c* [5,6]. In accordance with its light absorption spectrum [1], the red complex can thus be classified as a low-spin ferrihemochrome with one unpaired electron. The value obtained for the green complex is significantly higher, but still remains within the predicted limits of a low-spin compound [6]. The increase in the susceptibility, or part of it, could be due to a small fraction of unbound ferriheme. The susceptibility data do not offer any explanation for the spectral difference between the two complexes. It is further noteworthy that none of the complexes produced any detectable electron spin resonance absorption at the concentrations used when cooled to liquid nitrogen temperature.

MOLECULAR MODELS OF POLY-L-LYSINE:FERRIHEME

The ferrihemochrome nature of the red poly-L-lysine: ferriheme complex, which has now been determined by both spectrophotometric and magnetic measurements, requires specific octahedral coordination to the heme iron. The two non-porphyrin high field ligands involved are most likely ε-amino groups from the side-chains of the polypeptide. In the case of the green complex the structure of a low-spin hydroxide should also be considered, but preliminary data obtained with other polybases make this possibility unlikely (Blauer, unpublished data). Heme-heme interactions may play an important part in determining the properties of the green complex.



The binding of the porphyrin to both an α-helix and an open coil was investigated using models designed by Pauling and which were employed in an earlier investigation on cytochrome c [7]. These models were combined with Dreiding—Büchi stereomodels by means of special adapters. Both types of model were on the scale 0.4 Å per cm. Similarly, heme models consisting of metal plates of dimensions proportional to those obtained by X-ray diffraction on Ni-etioporphyrin [8] were adapted to the Büchi models at each of the 12 corners of the model plate. The appropriate side-chains were then fastened to the adapters. With regard to binding of the porphyrin to both right- and left-handed α-helices, three main different positions were considered in each case. If the heme disc is placed in a plane containing the helix axis and any two lysine side-chains are bound to the iron from opposite sides and perpendicular to the heme disc, there is indication of serious steric hindrance between the backbone of the helix and the methyl groups of other side-chains emerging from the porphyrin. Similarly, if the disc is bound in a plane perpendicular or at a slight inclination to the helix axis, the formation of such a chelate is rendered improbable, again because of steric hindrance (Fig. 1A). With the disc bound in a plane parallel to and at a suitable distance from the helix

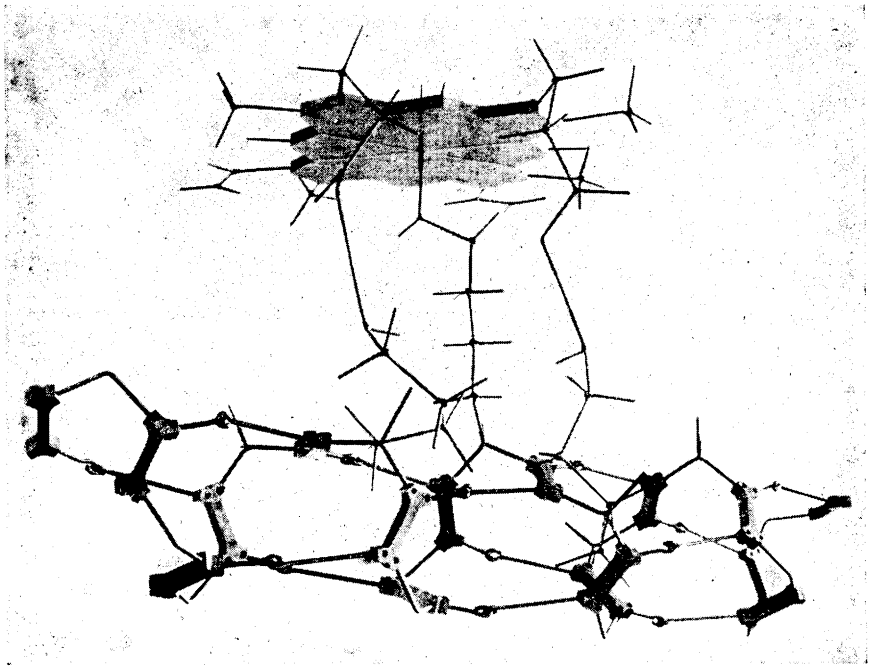


Fig. 1. Photographs of the constructed steric models of complexes between ferriheme and poly-L-lysine in the form of a righthanded α-helix, with the heme disc perpendicular to the helix axis (opposite page), and with the heme disc in a plane parallel to and at some distance from the helix axis (above).

axis, only one bond between an ε-amino group and the central iron atom can be formed and the other amino group must originate from another helix (Fig. 1B). In the latter case, for a given ε-amino-iron bond, many different combinations for interaction between the carboxylate side-chains of the porphyrin and charged ε-amino groups on the helix could be postulated. The two ironlinked ε-amino groups could also belong to the same polymer molecule, provided the helix were long enough and could bend back to allow the formation of a second bond.

Recent work on the optical rotatory dispersion of sperm whale myoglobin in aqueous solution [9] seems to confirm earlier correlations between the negative value of the optical rotatory dispersion parameter b_o and a right-handed α-helix. A negative value for b_o has, in fact, been determined for helical poly-L-lysine in aqueous solution [2]. In all the cases studied in the present work by means of steric models, the conclusions are essentially the same for both left- and right-handed helices.

When the porphyrin-iron of the model is bound to two neighbouring ε-amino groups of the unfolded peptide, there is indication of marked steric

hindrance. Except for large ring formation, bonding to a second chain would seem to be more likely in this case also.

As a consequence of the conclusions reached from the experiments with models, it seems likely that the mode of binding in the red complex is similar to that in ferricytochrome *c* [7]. Besides the bonds at the iron atom in both cases, the prosthetic group of the enzyme is also assumed to be linked to helical peptide by two thio-ether bonds, whereas in the synthetic complex interaction between the porphyrin carboxylates and protonated ε-amino groups of the polylysine is postulated. Work on the number of peptide and heme molecules in such ferriheme-polylysine aggregates is now in progress.

Acknowledgements. A.E. wishes to thank *Statens Medicinska Forskningsråd* for financial support. Thanks are due to professor Hugo Theorell for his kind hospitality extended to G.B. during his stay in Stockholm.

REFERENCES

1. Blauer, G. *Nature* **189** (1961) 396.
2. Applequist, J. B. *Thesis*, Harvard University 1959.
3. Theorell, H. and Ehrenberg, A. *Arkiv Fysik* **3** (1951) 299.
4. Ehrenberg, A. *Arkiv Kemi* **19** (1962) 119.
5. Boeri, E., Ehrenberg, A., Paul, K. G. and Theorell, H. *Biochim. Biophys. Acta* **12** (1953) 273.
6. Ehrenberg, A. *Svensk Kem. Tidskr.* **74** (1962) 103.
7. Ehrenberg, A. and Theorell, H. *Acta Chem. Scand.* **9** (1955) 1193.
8. Crute, M. B. *Acta Cryst.* **12** (1959) 24.
9. Urnes, P. J., Imahori, K. and Doty, P. *Proc. Natl. Acad. Sci. U.S.* **47** (1961) 1635.

Received June 8, 1962.