Effects of Formylation of Vinyl Side Chains of Heme on Optical and Ligand Binding Properties of Horse Heart Ferric Myoglobin*

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Effects of substitution of vinyl groups of hemin with formyl groups on the optical and ligand binding properties of horse heart ferric myoglobin were investigated. The peak positions as well as the line shapes of the absorption spectra of the ferric derivatives of three kinds of formylmyoglobin, 2-vinyl-4-formyl-, 2-formyl-4-vinyl-, and 2,4-diformylmyoglobins depend on the number and the position of the formyl groups. Absorption maxima in the Soret region of the acid forms of these ferric formylmyoglobins in 0.1 M potassium phosphate buffer, pH 6.0, at 20° were 415.2, 422, and 429 nm, respectively. The acid forms of these formylmyoglobins exhibit absorption spectra of the mixture of high- and low spin states at ambient temperature. Since proto-, deutero- and mesomyoglobins have a high spin state under the same condition, the increase of the low spin iron in these formylmyoglobins may be due to the strong electron withdrawal by the formyl groups toward the periphery of the porphyrin ring.

The affinities of these ferric formylmyoglobins and protomyoglobin for $N_3^-$, $F^-$, $OCN^-$, and $SCN^-$ increased in the order of proto-, monoformyl-monovinyl-, 2,4-diformyl-myoglobin, which corresponds to the increasing order of electron-withdrawing power of the porphyrin side chains. The $pK_a$ values of the acid-alkaline transition decreased in the same order.

Although the ferric forms of the two isomeric monoformyl-monovinylmyoglobins exhibited different optical spectra, the dissociation constants of the complexes of these isomers for various ligands were similar to each other. The $pK_a$ values of the acid-alkaline transition were also similar. These results indicate that affinities of ferric myoglobin for ligands, in contrast to those of the ferrous form for oxygen and carbon monoxide (Sono, M., and Asakura, T. (1975) J. Biol. Chem. 250, 5227-5232 and Sono, M., Smith, P. D., McCray, J. A., and Asakura, T. (1976) J. Biol. Chem. 251, 141-1426), are not affected by the position of modifications at the two vinyl groups, but are determined by the number of the formyl groups and that two vinyl groups at positions 2 and 4 are equivalent in the binding of various ligands by ferric myoglobin. The electron density of the ferric iron appears to be similar for the two isomeric monoformyl-monovinylmyoglobins.

The effects of formylation by chemical modifications (1) of the vinyl groups at positions 2 and 4 of the porphyrin ring on the optical and oxygen binding properties of the ferrous form of human hemoglobin (2) and horse heart myoglobin (3, 4) have been studied with respect to the equilibrium (2, 3) and kinetic (4) aspects. The formylation of the vinyl groups caused an optical red shift, a decrease in the intrinsic oxygen affinity, and an increase in both combination and dissociation rate constants. Most of these changes are attributed to the withdrawal of the $\pi$ electron density involved with oxygen binding toward the periphery of the porphyrin ring. It was also found that the two vinyl groups at positions 2 and 4 of ferrous heme side chains are nonequivalent in their optical, equilibrium and kinetic properties (2-4).

Heme modification studies of ferric hemoproteins have been reported for various hemoproteins including hemoglobin (5-7), myoglobin (8-11), horseradish peroxidase (12-14), and cytochrome c peroxidase (15, 16). Makino and Yamazaki (11, 12) indicated that the $pK_a$ values of the dissociation constants of the complexes with various ionic ligands and the $pK_a$ values of the transition between the acid and alkaline forms for the reconstituted horseradish peroxidase or sperm whale myoglobin have a close correlation with $pK_a$ values of the metal-free porphyrins.

\[ pK_a = pH - \log\left(\frac{PH_a}{PH_{a+}}\right), \]  where $PH_a$ and $PH_{a+}$ are neutral and monocationic species of the porphyrin, respectively (20).
All of these studies, however, were made without giving special attention to whether the two vinyl groups of hemin are functionally equivalent.

The present paper deals with the effects of heme substitution on optical and equilibrium ligand binding properties of horse heart ferric myoglobins containing two isomers of monoformal- monovinylhemin, 2,4-diformyl-, proto-, deutero-, and mesohemin.

**EXPERIMENTAL PROCEDURE**

**Myoglobins**

Native protomyoglobin was obtained from horse heart muscle by the method of Yamazaki et al. (17). Apomyoglobin was prepared by treating ferric myoglobin with HCl-methyl ethyl ketone (18, 19). Various reconstituted myoglobins were prepared as described previously (3) and followed by purification on DEAE-cellulose column chromatography (13). Since formylmyoglobins were found to be partially reduced to the oxy form (<10%) during recombination procedures, they were completely oxidized to the ferric form by addition of a small amount of potassium ferricyanide and passed through a Sephadex G-25 column.

**Hemins**

Three kinds of formyl heme were prepared by a method described elsewhere (1, 2). The preparations of deutero- and mesohemins were made according to the methods of Chu and Chu (20) and Fischer and Pützer (15, 21), respectively. A standard technique (13) was used for the incorporation of iron into these porphyrins.

**Reagents**

All chemicals of reagent grade were purchased from the following companies and used without further purification: potassium phosphate, potassium cyanide, potassium cyanate, and potassium thiocyanate from Fisher Scientific Co.; sodium azide from Matheson Coleman and Bell Co.; potassium fluoride from J T Baker Chemical Co.; and sodium fluoride from Matheson Coleman and Bell Co.

**Acid-Alkaline Transition Measurements**

The $pK_a$ value of the transition between the acid and alkaline forms of ferric formylmyoglobin was determined spectrophotometrically in both visible and Soret regions and was calculated from the following equation, assuming that $n = 1$,

$$pK_a = n \frac{1}{pH - \log \left( \frac{[\text{alkaline form}]}{[\text{acid form}]} \right)}$$

where $n$ is the Hill constant. The measurements were made at the wavelengths of the peak positions of acid forms in the Soret region (Table I) and those of the alkaline forms in the visible region, 593, 598, and 602 nm for 2-vinyl-4-formyl-, 2-formyl-4-vinyl-, and 2,4-diformylmyoglobin, respectively. A small amount of properly diluted NaOH solution was added successively to the ferric myoglobin solution containing 10.5 to 16.5 $\mu$g myoglobin in 2 $\mu$L phosphate solution adjusted to pH 6. The pH and the spectral changes were recorded at each addition of NaOH solution.

**Ligands Titration**

The equilibrium dissociation constants ($K$) of the various ferric myoglobin-ligand complexes were obtained by measuring the absorbance changes of the ferric myoglobins upon successive additions of various ligands and were determined by Hill plots expressed by the following equation, assuming that $n = 1$,

$$\log Y(1 - Y) = n \log \left( \frac{[\text{ligand}]}{[\text{ligand}]} \right) + a$$

where $Y$ and $n$ are the fractional formation of ligand complex and slope in Hill plot for complex formation, respectively, and $a$ is a constant. Since $pK_a$ value ($K = (1 - Y) (\text{ligand})/Y$) is given by $-\log (\text{ligand})$ with $Y = 0.5$ the value $pK_a$ is calculated to be $pK_a = a/n$.

**Other Methods**

**Spectrophotometric Measurements**—Measurements were carried out with a Perkin-Elmer Coleman 124 spectrophotometer equipped with a temperature control.

**RESULTS**

**Absorption Spectra**—Absorption spectra of three kinds of reconstituted ferric formylmyoglobin in the acid forms and their complexes with $-\text{OH}^-$, $\text{CN}^-$, $\text{N}_3^-$, $\text{SCN}^-$, and $F^-$ are shown in Fig. 1. A to F. Corresponding spectra of ferric protomyoglobin are also shown for comparison. The absorption maxima (nanometers) and the extinction coefficients of the ferric derivatives of three kinds of formylmyoglobin were summarized in Table I. The line shapes in the visible region of most of these myoglobins for each ligand show considerable differences even between the spectra of the two isomeric monoformal-monovinylmyoglobins. Therefore, it is difficult to correlate the visible spectrum with the electron attractivity of heme side chains, as has been done for the oxy forms (2, 3). If we compare the Soret peaks of these myoglobins, they clearly show increasing red shifts in the order of proto-, monoformal-monovinyl, and 2,4-diformylmyoglobin. This order is consistent with the increasing electron-withdrawing power of the porphyrin side chains. The two isomeric myoglobins containing monoformal-monovinylhemin exhibit differences not only in the shape of the visible spectra, but also in the peak positions in the Soret region; the peak positions of 2-formyl-4-vinylmyoglobin derivatives shifted to the longer wavelengths than those of 2-vinyl-4-formylmyoglobin derivatives by 4.5 to 7.0 nm.

Since the optical properties of the free forms of these two isomeric hemes are similar (2), differences observed after recombination with apomyoglobin must be caused by the interaction with protein. Spectra of the acid forms, and cyanide and thiocyanate complexes of three formylmyoglobins were of high spin type having the so-called charge transfer bands around 510 and 660 nm, and those of alkaline forms and azide and cyanate complexes were of low spin type showing the main bands around 560 and 600 nm. The spectra of fluoride complexes of three formylmyoglobins are complicated. Those of monoformal-monovinylmyoglobins were similar in shape to their corresponding alkaline forms and the spectrum of fluoride complex of 2,4-diformylmyoglobin was similar to the alkaline form of protomyoglobin.

**Acid-Alkaline Transition**—A typical result of spectrophotometric pH titration for 2-vinyl-4-formylmyoglobin is shown in Fig. 2. In each titration one can see clear isosbestic points between pH 6 and 10. The Hill plots of the titrations calculated from the absorbance changes are shown in Fig. 3 together with the result for reconstituted protomyoglobin reported by Tamura et al. (10). The titration curves measured at the Soret and visible regions gave identical results. The slope of the Hill plots is unity for all myoglobins. The $pK_a$ values are decreased in the order of proto- monoformal-monovinyl, 2,4-diformylmyoglobin, indicating the acidity of the water molecule bound to the ferric iron, i.e. ionizability of the water molecule, increases in the same order as above, which is consistent with the increasing electron-withdrawing power of the porphyrin side chains. In contrast to the ligand binding properties of the ferrous forms (3), the two isomeric myoglobins containing 2-formyl-4-vinyl- and 2-vinyl-4-formylhemin show very close $pK_a$ values (7.95 and 8.02, respectively), indicating that the effects of formylation of the two vinyl groups at positions 2 and 4 are almost equivalent in the acid–alkaline transition of ferric myoglobin.

**Azoide Titrations**—Fig. 4 shows optical absorption changes of...
Fig. 1. Absorption spectra of ferric formylmyoglobins and their complexes with various ligands: acid forms (A), alkaline forms (B), cyanide complexes (C), azide complexes (D), fluoride complexes (E), and thiocyanate complexes (F). Each set of spectra shows the optical absorption spectra of proto-, 2-vinyl-4-formyl-(2-V-4-F), 2-formyl-4-vinyl-(2-F-4-V), and 2,4-diformylmyoglobins (2,4-DiF) from the top to the bottom in this order. The spectra were measured in 0.1 M potassium phosphate buffer, pH 6.0, at 20°C, except for those of the alkaline forms which were measured in 0.1 M glycine/NaOH buffer, pH 10.0, at 20°C. The concentrations of the myoglobins were between 10.0 and 16.5 μM.
### Properties of Ferric Myoglobins Containing Formylhemes

**Table I**

| Myoglobin | pH | Light Absorption Maxima (nm) |
|-----------|----|-----------------------------|
| 2-formyl-4-vinyl-Mb<sup>(H<sub>2</sub>O)</sup> | 6.0 | 281(37.1) 422(109) 510(10.0) 550(8.76) 602(5.10) 655(3.28) |
| 7.0 | 282(36.7) 423(109) 509(10.1) 550(8.85) 600(5.54) 656(3.28) |
| 10.0 | 280(37.9) 552(9.18) 596(9.20) 636(6.92) |
| Mb<sup>+</sup>F<sup>-</sup> | 6.0 | 426(95.4) 450(9.97) 504(9.37) 548(9.78) 594(9.66) 644(3.27) |
| 7.0 | 416(117) 446(104) 490(10.4) 549(9.48) 593(9.48) 645(3.27) |
| Mb<sup>+</sup>N<sub>3</sub> | 6.0 | 435(95.4) 465(9.97) 519(9.37) 573(9.78) 623(9.66) 673(3.27) |
| 7.0 | 425(109) 455(104) 509(10.4) 569(9.48) 619(9.48) 669(3.27) |
| Mb-CN<sup>-</sup> | 6.0 | 429(93.9) 459(9.97) 513(9.37) 567(9.78) 617(9.66) 667(3.27) |
| 7.0 | 419(102) 449(104) 503(10.4) 553(9.48) 603(9.48) 653(3.27) |
| Mb+SCN<sup>-</sup> | 6.0 | 425(93.9) 455(9.97) 513(9.37) 567(9.78) 617(9.66) 667(3.27) |
| 7.0 | 415(102) 445(104) 503(10.4) 553(9.48) 603(9.48) 653(3.27) |
| Mb+OCN<sup>-</sup> | 6.0 | 429(93.9) 459(9.97) 513(9.37) 567(9.78) 617(9.66) 667(3.27) |
| 7.0 | 419(102) 449(104) 503(10.4) 553(9.48) 603(9.48) 653(3.27) |
| Mb+OH<sup>-</sup> | 6.0 | 429(93.9) 459(9.97) 513(9.37) 567(9.78) 617(9.66) 667(3.27) |
| 7.0 | 419(102) 449(104) 503(10.4) 553(9.48) 603(9.48) 653(3.27) |

*Wavelength (λ) is given in nanometers; the molar absorption coefficients (mM⁻¹cm⁻¹) are indicated in parentheses.

**Fig. 2.** Optical transition between the acid and alkaline forms of ferric 2-vinyl-4-formylmyoglobin. The myoglobin concentration was 15.3 μM. The measurements were made in 2 mM potassium phosphate solution at 20°C. The scale of the optical density at the Soret region is 10 times larger than that of the visible region.

**Fig. 3.** The Hill plots of the formation of the alkaline forms of ferric 2,4-diformyl- (Δ), 2-formyl-4-vinyl- (○), 2-vinyl-4-formyl- (△), and proto- (---) myoglobins. The measurements were carried out at 20°C as described in the text. The values of ferric protomyoglobin were quoted from the result reported by Tamura et al. (10).

Cyanide Titration—Although optical absorption changes which occur upon successive additions of cyanide showed a single set of isosbestic points for all myoglobins, the Hill plots of these titrations did not show straight lines. A similar result was reported by Scheler and Jung (22).

It took more than 20 min to reach an equilibrium after each addition of cyanide to myoglobin, while the other ligands required less than 3 min. Since the Hill plots of the formation of the cyanide complexes of various myoglobins did not exhibit uniform curves, and the n-values were not of unity, accurate determinations of the dissociation constants of cyanide for myoglobins were not possible.
Properties of Ferric Myoglobins Containing Formylhemes

**Fluoride Titration**—The Hill plots for the formation of fluoride complexes for various myoglobins (Fig. 5B) indicated that the affinity for fluoride is increased in the order of deutero-, meso-, protoformyl-monovinyl-, 2,4-diformylmyoglobin. The magnitude of affinity for fluoride was decreased by the factor of 300 compared with that of affinity for azide for all myoglobins studied in this experiment. 2-Formyl-4-vinylmyoglobin showed about 30% higher affinity for fluoride than 2-vinyl-4-formylmyoglobin at pH values of 6.0 and 7.0.

**Cyanate and Thiocyanate Titrations**—The Hill plots for cyanate and thiocyanate complexes formation are shown in Fig. 5, C and D. The slope for both complexes was 1 for all myoglobins examined. The affinities for cyanate and thiocyanate decreased in the order 2,4-diformyl-, monofomyl-monovinyl-, protomyoglobin. However, meso- and deuteroformylmyoglobins exhibited similar affinities for these ligands to that of protomyoglobin. The affinities of 2-formyl-4-vinylmyoglobin for both ligands were slightly higher (15 to 25%) than those of 2-vinyl-4-formylmyoglobin.

**Effects of pH and Hemin Substitution on Dissociation Constants (K)**—The pKₐ and pK values of various myoglobins for several ionic ligand complexes are summarized in Table II. In all cases affinities (pK) for fluoride, thiocyanate, and azide at pH 6.0 were higher than at pH 7.0. As shown in Fig. 6, all myoglobins exhibited increasing ligand affinities with the increase of electron-withdrawing power of the porphyrin side chains, except for the affinities of deutero- and mesomyoglobins for fluoride, cyanate, and thiocyanate.

**DISCUSSION**

**Properties of Two Isomeric Monoformyl-monovinylmyoglobins in Ferric Form**—Significant differences between the absorption spectra of the two isomeric monoformyl-monovinyl ferric myoglobins were observed for all liganded states examined. The order and magnitude of the optical red shifts for the three formylmyoglobins were similar to those for the ferrous states.

**Relation between pKₐ of Porphyrin and Ligand Affinity of Ferric Myoglobin**—The close relationship between the electron-withdrawing power of the substituents at positions 2 and 4 of the porphyrin ring and the change in the reactivities of the ferric forms of horseradish peroxidase and sperm whale myoglobin were reported by Makino and Yamazaki (11, 12). Similar relationships were observed for horse heart myoglobin between the pKₐ of the acid-alkaline transition or pK of the azide complex formation and pKₐ of the metal-free porphyrins (Fig. 6.). The dissociation constants (K) for the other ligands,
Properties of Ferric Myoglobins Containing Formylhemes

Dissociation constants for equilibrium reactions between reconstituted ferric myoglobins and various ionic ligands

Measurements were made in 0.1 M potassium phosphate buffer at 20°C. The concentrations of the myoglobins were 10 to 15 μM for native and formylmyoglobins, and 5 to 9 μM for the other reconstituted myoglobins.

| Myoglobin | Fluoride complex | Thiocyanate complex | Cyanate complex | Azide complex |
|-----------|------------------|---------------------|----------------|---------------|
|           | 6.0  | 7.0  | 6.0  | 7.0  | 1/1  | 6.0  | 7.0  | 1/1  |
| 2,4-diformyl- | Kx10^5 (pK_x)   | 0.6(1.24)         | 1.1(1.71)      | 0.14(3.88) | 0.28(3.55) | 0.60(3.22) | 2.1(3.88) | 2.2(3.80) |
| 2-formyl-4-vinyl- | 6.0  | 7.0  | 6.0  | 7.0  | 1/1  | 6.0  | 7.0  | 1/1  |
| 2-vinyl-4-formyl- | 6.0  | 7.0  | 6.0  | 7.0  | 1/1  | 6.0  | 7.0  | 1/1  |
| Proto(M) | 6.9(2.16) | 14.5(1.84) | 2.04(2.69) | 3.80(2.42) | 2.63(2.58) | 20.4(4.69) | 38.0(4.42) |
| Proto(Reconst) | 6.3(2.17) | 14.5(1.84) | 2.04(2.69) | 3.80(2.42) | 2.63(2.58) | 20.4(4.69) | 38.0(4.42) |
| Deutero- | 12.9(1.89) | 24.5(1.61) | 2.63(2.58) | 6.07(2.39) | 2.63(2.58) | 20.4(4.69) | 38.0(4.42) |
| Meso- | 10.2(1.99) | 19.1(1.72) | 1.26(2.90) | 1.62(2.76) | 2.63(2.58) | 39.8(4.40) | 51.3(4.29) |

Phosphate, 20°C

Fig. 6. Plots of pK (K, dissociation constant) for the complexes of the ferric myoglobins with various ligands against pK_a of the corresponding metal-free porphyrins. The pK values were taken from Table I. The pK_a values are referred to Table II in ref. 23. The scale for the pK_a values is graduated on the right ordinate. The plots are straight lines (Fig. 6). Although the reason for this deviation is not clear, it may be related to the thermal equilibrium state of the ferric heme iron. As mentioned below, the acid form of ferric formylmyoglobin exhibits a thermal mixture of the high spin state and low spin state spectra (24) at room temperature, whereas those of ferric proto-, deutero-, and mesomyoglobins show the typical high spin spectra (25-27).

Optical and Spin State Properties of Ferric Formylmyoglobins—The substitution of vinyl groups by formyl groups significantly changes the optical properties of ferric myoglobins. The acid forms of the three ferric formylmyoglobins give the four-banded spectra of a mixed type in the visible region at pH 6.0 (Fig. 1A). Two peaks at about 510 and 660 nm of these four peaks are the so-called charge transfer bands and are specific to the high spin state. The other two peaks at about 660 and 600 nm appear close to the wavelengths where the alkaline form of ferric myoglobin has its maxima and are attributed to the spectra of the low spin state. Since there is no spectral change when pH is lowered from 6 to 5, the acid form spectra of the formylmyoglobins are undoubtedly those of a mixture of high and low spin forms, which may be induced by the strong electron-attractive formyl groups at the periphery of the porphyrin ring. Iizuka and Orii (24) reported that at a low temperature of 89 K, the acid form of chlorocruoromyoglobin (complex of sperm whale apomyoglobin and 2-formyl-4-vinyldeuterohemin) in Tris-HCl buffer, pH 7.0, exhibited the high spin type of spectra with less distinct bands at 550 and 600 nm, which are due to the low spin state form. However, the acid form of the other reconstituted myoglobins with less electron-attractive side chains, such as meso- and deuteror derivatives, have typical high spin-type spectra similar to that of protomyoglobin (9). The dissociation constants of meso-, deutero-, and protomyoglobins for the complexes of fluoride, cyanate, and thiocyanate, which are of high spin types, are similar to one another, whereas the constants of the formylmyoglobins vary according to the pK_a values of the porphyrins. Because such deviation is not found when the ligands are azide and hydroxyl ion, which form the complexes of the low spin state, there may exist a certain correlation between the spin state and the ligand affinity of ferric hemoprotein.

REFERENCES
1. Sono, M., and Asakura, T. (1974) Biochemistry 13, 4386-4394
2. Asakura, T., and Sono, M. (1974) J. Biol. Chem. 249, 7087-7093
3. Sono, M., and Asakura, T. (1976) J. Biol. Chem. 250, 5227-5232
4. Sono, M., Smith, P. D., McCoy, J. A., and Asakura, T. (1976) J. Biol. Chem. 251, 1418-1426
5. Antonini, E., Brunori, M., Caputo, A., Chiaveggio, E., Rossi, E., Fanelli, A., and Wyman, J. (1964) Biochim. Biophys. Acta 79, 284-292
6. Brunori, M., Amicucci, G., Antonini, E., Wyman, J., Zito, R., and...
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Rossi Fanelli, A. (1968) Biochim. Biophys. Acta 154, 315-322
7. Asakura, T., Minakami, S., Yoneyama, Y., and Yoshikawa, H. (1964) J. Biochem. (Tokyo) 56, 594-600
8. O’Hagan, J. E., and George, P. (1960) Biochem. J. 74, 424-427
9. Tamura, M., Asakura, T., and Yonetani, T. (1973) Biochim. Biophys. Acta 295, 467-479
10. Tamura, M., Woodrow, G. V., III, and Yonetani, T. (1973) Biochim. Biophys. Acta 317, 34-49
11. Makino, R., and Yamazaki, I. (1973) Arch. Biochem. Biophys. 157, 350-368
12. Makino, R., and Yamazaki, I. (1972) J. Biochem. (Tokyo) 25, 655-664
13. Tamura, M., Asakura, T., and Yonetani, T. (1972) Biochim. Biophys. Acta 264, 292-304
14. Ohlsson, P. I., and Paul, K. G. (1973) Biochim. Biophys. Acta 315, 293-305
15. Yonetani, T., and Asakura, T. (1968) J. Biol. Chem. 243, 4715-4721
16. Asakura, T., and Yonetani, T. (1969) J. Biol. Chem. 244, 4573-4579
17. Yamazaki, I., Yokota, K., and Shikama, K. (1964) J. Biol. Chem. 239, 4151-4155
18. Yonetani, T., and Asakura, T. (1969) J. Biol. Chem. 244, 4580-4588
19. Teale, F. W. J. (1959) Biochim. Biophys. Acta 35, 543
20. Chu, T. C., and Chu, E. J. H. (1952) J. Am. Chem. Soc. 74, 6276-6277
21. Fischer, H., and Pützer, B. (1926) Hoppe-Seyler's Z. Physiol. Chem. 154, 39-63
22. Scheler, W., and Jung, F. (1958) Acta Biol. Med. Germ. 1, 232-235
23. Falk, J. E. (1964) Porphyrins and Metalloporphyrins, pp. 26-29, Elsevier Publishing Co., Amsterdam
24. Iizuka, T., and Orii, Y. (1973) Biochim. Biophys. Acta 328, 275-286
25. Theorell, H., and Ehrenberg, A. (1951) Acta Chem. Scand. 5, 823-848
26. George, P., Beetlestone, J., and Griffith, J. S. (1964) Rev. Mod. Phys. 36, 441-459
27. Iizuka, T., and Kotani, M. (1969) Biochim. Biophys. Acta 181, 275-286
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