and since the effects of the epoxy functions in this reaction are unknown the possibility that these compounds contain hydroxymethyl groups cannot be completely ruled out. The weight of available evidence, however, suggests that β-carotene, lutein, violaxanthin, and neoxanthin have been correctly identified by the previous workers.[1-3]

Culturing conditions [14] and chemical methods [15] have been described elsewhere.

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Crystalline Leghemoglobin

XIV. Transfer of Hematin from Lb*a* and Lb*c* to Horse-radish Peroxidase Apoprotein

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In 1960 Rossi-Fanelli and Antonini noted the transfer of hematin from *Aplysia* methemoglobin to horse apomyoglobin.[1] Until then the bond between heme and globin had been considered very stable under physiological conditions. A similar migration of hematin has also been shown to take place between some other hemoproteins, *e.g.* myoglobin and horse-radish apoperoxidase (apoHRP).[2] In the present investigation the transfer of hematin from the fast and slow components of soybean leghemoglobin, Lb*a* and Lb*c*, to apoHRP has been studied. The appearance of the peroxidase activity is taken as evidence for the correct binding of hematin to apoHRP. For comparison, the transfer of the hematin of horse myoglobin (Mb I) to apoHRP was also studied.

Materials and methods. Lb*a*, Lb*c* and yeast cytochrome *c* peroxidase (YCCP) were prepared as described previously.[3,4] Horse heart cytochrome *c* was a commercial preparation (Type III) from Sigma Chemical Company (St. Louis, U.S.A.). Horse myoglobin (Mb I) was isolated according to Åkeson and Theorell.[5] Horse radish peroxidase, component C (HRP C) was prepared according to Paul,[6] and its apoprotein according to Theorell and Maehly.[7] The heme-binding capacity of apoHRP was determined by titration with hematin. The peroxidase activity was assayed by the guaiacol method.[8] The concentration of hydrogen peroxide was determined enzymatically according to Yonetani.[9] ApoHRP and leghemoglobin or myoglobin, respectively, were incubated in 0.02 M sodium phosphate buffer, pH 7.0, for 18 h at 25°C. The peroxidatic activity of the solution was measured and the amount of HRP formed calculated.

Results and discussion. Fig. 1 portrays the transfer of hematin from Lb*a*, Lb*c* and Mb I to apoHRP at pH 7.0. The values obtained were corrected for the inherent

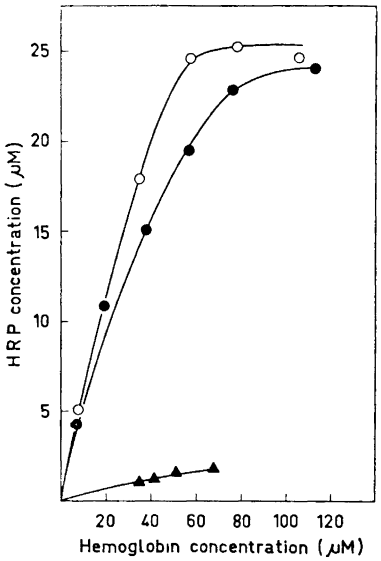

Fig. 1. The formation of HRP from apoHRP and Lb*a* (○), Lbc (●) and MbI (▲) in 0.02 M sodium phosphate buffer, pH 7.0. Ordinate: HRP formed as calculated from the activity measurements; abscissa: concentration of hemoglobin in incubation solution.

peroxidatic activity of Lb*a*, Lbc, Mb I, and apoHRP. The activity obtained is therefore a consequence of the formation of holoHRP.

Banerjee [10] has defined the equilibrium constant for the dissociation of a hemoprotein to yield dimeric hematin and protein and derived the value $\log K = 15.24$ at 25°C for metmyoglobin at pH 7.0. A comparison of the affinities of the apoproteins of Lb*a*, Lbc, and Mb I allows a relative value of 12.81 for Lb*a* and 12.91 for Lbc to be estimated for the hematin-protein association constant, assuming the constant for Mb I to be equal to the above-mentioned 15.24.

These findings present a new case of hematin transfer between proteins. Under the conditions studied (pH 7.0) heme is about 370 times more firmly bound to the apoprotein in horse Mb I than in Lb*a*, and 250 times more firmly than in Lbc. A comparative study on urea denaturation of Lb*a*, Lbc and sperm whale myoglobin indicates that sperm whale myoglobin is more stable to urea denaturation than the two leghemoglobin components.[11] It appears therefore, as through the "heme pocket" of Lb*a* and Lbc is more open than that of myoglobin, and allows an easier migration of the heme.

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On the Nonsymmetry of Reaction Parameter (ϱ) and the Substituent Parameter (σ) in the Hammett Equation and Similar Extrathermodynamic Relationships

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The Hammett equation (1) has empirically been found to well describe aromatic

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