Kinetics of the Incorporation of Magnesium(II) into Porphyrin[1]

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Abstract: Kinetics of the formation of magnesium deuteroporphyrin dimethyl ester were measured in acetone and methanol as solvents. The reaction was found to be catalyzed by pyridine and certain other nitrogenous bases. Pyridine was found also to aid in the removal of both Mg and Zn from their respective metalloporphyrins. In methanol, the rate law was found to be $d[MgP]/dt = k_1[PH_2]/[H^+] + k_2[PH_2][Mg][\text{pyridine}]/[H^+]$, where PH_2 denotes free porphyrin ester and $k_1 = 1.3 \times 10^{-7}\ M\ \text{sec}^{-1}$ and $k_2 = 1.0 \times 10^{-3}\ M^{-1}\ \text{sec}^{-1}$. The apparent activation energies for k_1 and k_2 were found to be 36.5 and 15.8 kcal/mole, respectively. The rate law is discussed in terms of a dissociation path plus a displacement path involving a magnesium-pyridine-porphyrin ternary complex.

In an earlier publication[2] it was reported that the incorporation of Mg(II) into porphyrins in the presence of water had been carried out for the first time by taking advantage of the observed catalysis by pyridine and 2,4-lutidene. Although most experiments were conducted with deuteroporphyrin dimethyl ester, the esters of hemoto-, proto-, meso-, and coproporphyrin behaved similarly. Thus, the catalysis seems quite general and may well be involved in the biosynthesis of chlorophyll. The present study was undertaken to measure the kinetics of the Mg(II) incorporation in order to elucidate the mechanism of the reaction and to attempt to explain the reason why Mg(II) incorporation is found to be so slow relative to other metals. Fleischer has established the relative rates of metal-ion incorporation to be Cu(II) > Zn(II) > Mn(II) > Co(II) > Fe(II) > Ni(II) > Cd(II).[3] It is generally found that Mg(II) is slower than any of the ions listed. Until the discovery of the pyridine catalysis,[2] it was not possible to incorporate Mg(II) except under strictly anhydrous conditions. Certainly the unique position of Mg(II) like the kinetic order of other metal ions is not accounted for by simple considerations such as ionic radii, gross electronic structure, etc.

Experimental Section

All spectra were taken with either a Beckman D.B. or a Bausch and Lomb 505 recording spectrophotometer. The kinetics were carried out in a thermostated bath with a maximum temperature variation of ±0.02°. All pH measurements were made with a Leeds & Northrup pH meter; the readings were all temperature corrected by using the adjustment on the instrument.

Deuteroporphyrin was prepared by essentially the method of Chu and Chu.[4] Absorption maxima of the product in $CHCl_3$ were at 620, 566, 530, and 498 mμ and a Soret band at 400 mμ. Extinction coefficients were 2.78×10^3, 4.58×10^3, 6.15×10^3, 11.1×10^3, and 1.35×10^5, respectively. Magnesium salts used throughout the study were the purest grades commercially available. Pyridine was dried over NaOH pellets and distilled. Acetone was dried over anhydrous K_2CO_3, filtered, and distilled. Analytical grade, anhydrous methanol was dried over CaO and distilled. Tris buffers, $(CH_2OH)_3CNH_2$ plus $(CH_2OH)_3CNH_3Cl$, were Sigma Chemical Co., reagent grade. The temperature coefficient of pH for the several buffer mixtures was supplied by the manufacturer, checked and extended by direct measurement. Identical pH readings for the Tris buffers were found for aqueous solution, methanol solution, and mixtures of the two solvents. For this reason, empirical pH values are reported for methanol solutions as being the ones measured directly.

Kinetic experiments were performed by preparing 20-ml solutions of the desired concentration of reagents and sealing 3-ml aliquots in glass ampoules which were placed in an oil bath thermostated at 100°. At predetermined time intervals (usually 5 or 10 min), an ampoule was quenched in ice-water and opened, and a difference spectrum was measured *vs.* the original solution (no reaction detectible in the original, cold solution). The absorption increase at the Soret band of the magnesium-porphyrin was found to be most sensitive to the extent of reaction. In all cases the initial rise was linear with time within the small random scatter of the five data points measured for each kinetic run. The resulting uncertainty in slope was less than 5% for each experiment. The initial rates so measured were in good agreement with apparent first-order rate constants obtained for longer reaction times in solutions where all reagents but one were in large excess.[5]

Results

Kinetics in Acetone. Studies were conducted on the over-all reaction

$$Mg^{2+} + PH_2 \longrightarrow MgP + 2H^+$$

(PH_2 indicates the neutral molecule deuteroporphyrin dimethyl ester) in acetone where true solutions are formed as indicated by Beer's law behavior (within 1% throughout the concentration range of $PH_2 = 1$ to $50 \times 10^{-6}\ M$ in both acetone and methanol). The reaction is extremely slow even at 100°, but could be carried to completion if the acetone solvent was allowed to evaporate completely. The reaction was found to be catalyzed significantly by a variety of bases including pyridine, 2,4-lutidene, N-methylaniline, imidazole, β-picoline, isoquinoline, and OH^- ($10^{-6}\ M$). Some catalysis was observed for pyrrole, 2,2-bipyridine, 2,2,2-terpyridine, piperidine, quinoline, and 2-amino-4-methylpyrimidine. No appreciable catalysis was detectable for 2,6-lutidene, N,N-dimethylformamide, ethylenediamine, triethylamine, *n*-butylamine, diphenylamine, 2,4-dinitroaniline, and phenol. Pyridine was chosen as the catalyst for the detailed kinetic investigations. As the source of Mg(II), $Mg(ClO_4)_2$ was chosen since it should be the best source of uncomplexed Mg(II). Experiments with $MgCl_2 \cdot 6H_2O$ and

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[1] From the Ph.D. Thesis of S. J. Baum; supported by Molecular Biology Training Grant 5T1GM834, National Institutes of Health, USPHS, and by Research Grant GM11788 from the National Institutes of Health, USPHS.

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[3] E. B. Fleischer, E. I. Choi, P. Hambright, and A. Stone, *Inorg. Chem.*, **3**, 1284 (1964).

[4] T. C. Chu and E. Chu, *J. Am. Chem. Soc.*, **74**, 6276 (1952).

[5] S. J. Baum. Thesis, Cornell University, 1965.

Table I. Kinetics in Acetone at 100°

Concentrations, M Porphyrin	Mg(II)	Pyridine	Initial rate, M/sec	Rate/ [PH$_2$][Mg][pyr]
1.3×10^{-5}	5.4×10^{-3}	6.2×10^{-2}	4.05×10^{-4}	9.3×10^{4}
6.5×10^{-6}	5.4×10^{-3}	6.2×10^{-2}	1.94×10^{-4}	8.9×10^{4}
3.3×10^{-6}	5.4×10^{-3}	6.2×10^{-2}	1.00×10^{-4}	9.2×10^{4}
1.3×10^{-5}	2.7×10^{-3}	6.2×10^{-2}	1.96×10^{-4}	9.0×10^{4}
1.3×10^{-5}	1.4×10^{-3}	6.2×10^{-2}	9.56×10^{-5}	8.8×10^{4}
6.5×10^{-6}	5.4×10^{-3}	2.5×10^{-2}	1.50×10^{-4}	1.7×10^{5}
6.5×10^{-6}	5.4×10^{-3}	1.2×10^{-2}	9.92×10^{-5}	2.4×10^{5}
6.5×10^{-6}	5.4×10^{-3}	6.2×10^{-3}	7.58×10^{-5}	3.5×10^{5}
6.5×10^{-6}	5.4×10^{-3}	3.1×10^{-3}	5.00×10^{-5}	4.6×10^{5}
1.3×10^{-5}	2.2×10^{-2}	6.2×10^{-4}	1.08×10^{-4}	6.1×10^{5}
1.3×10^{-5}	2.2×10^{-2}	3.1×10^{-4}	7.00×10^{-5}	7.8×10^{5}
1.3×10^{-5}	2.2×10^{-2}	1.6×10^{-4}	4.66×10^{-5}	1.0×10^{6}
1.3×10^{-5}	2.2×10^{-2}	9.9×10^{-5}	4.58×10^{-5}	1.6×10^{6}
1.3×10^{-5}	2.2×10^{-2}	7.8×10^{-5}	4.36×10^{-5}	2.0×10^{6}
1.3×10^{-5}	2.2×10^{-2}	3.9×10^{-5}	4.03×10^{-5}	3.6×10^{6}
1.3×10^{-5}	2.2×10^{-2}	2.5×10^{-5}	3.85×10^{-5}	5.4×10^{6}
1.3×10^{-5}	2.2×10^{-2}	1.9×10^{-5}	3.33×10^{-5}	6.1×10^{6}

$Mg(NO_3)_2 \cdot 6H_2O$ showed slower rates, and $Mg(C_2H_3O_2)_2 \cdot 4H_2O$ showed rates that were even slower.

Table I summarizes the results of the kinetic experiments in acetone at 100° with pyridine as catalyst. For purposes of comparison, the last column in the table contains the value obtained by dividing the observed initial rate by the concentrations of porphyrin, Mg(II), and pyridine. It is seen from the first five entries (pyridine constant) that the reaction is first order in porphyrin and first order in Mg(II). However, with changed pyridine concentration, the rate does not change proportionally. At high pyridine the rate is about half-order, and at lower pyridine it is less than this. The complexity with respect to pyridine might result from formation of significantly large concentrations of magnesium(II)–pyridine complexes. However, experiments (spectral, and measurement of pyridine concentrations in the vapor phase by both mass spectrometry and vapor phase chromatography) failed to show appreciable concentrations of complexed pyridine. An alternate explanation for the nonsimple pyridine dependence is that the reaction is sensitive to pH changes brought about by changing solution concentrations. Attempts to buffer the solutions failed, and it was found necessary for further investigation to change solvents.

Kinetics in Methanol. Various solvents for the reaction were investigated to find one which would dissolve porphyrin, $Mg(ClO_4)_2$, pyridine, and Tris buffer. Methanol was found suitable. Table II presents the data for buffered solutions in methanol. The H^+ concentrations are given as those for the corresponding buffer in aqueous solution since direct measurement with a glass electrode gave the same values for both solvents. At the H^+ concentrations listed, the reaction proceeds at a measurable rate without catalyst. From the experiments in the absence of catalyst, the rate is seen to be proportional to porphyrin concentration, independent of Mg(II) concentration, and inverse in H^+ concentration. The values listed for k_1 refer to the expression

$$\frac{d[MgP]}{dt} = k_1\frac{[PH_2]}{[H^+]}$$

In the presence of the catalyst pyridine, the rate of the catalyzed path is directly proportional to both Mg(II), porphyrin, and pyridine concentrations, and inverse in H^+ concentration. This can be seen by comparing the values calculated for k_2 which refers to the complete rate law

$$\frac{d[MgP]}{dt} = k_1\frac{[PH_2]}{[H^+]} + k_2\frac{[PH_2][Mg][pyr]}{[H^+]}$$

Apparent activation energies for the two paths in the rate expression were determined by running additional kinetic experiments at both lower (90°) and higher temperature (110°). Changes of temperature caused slight changes of pH (7.4 at 90°, 7.5 at 100°, 7.6 at 110°) which were taken into account. When the first experiment of Table II was run at 90°, the initial rate was found to be 2.1×10^{-5} M/sec, and at 110° it was 2.1×10^{-4}. From the corresponding rate constants, an apparent activation energy of 36.5 ± 0.5 kcal/mole was found for the uncatalyzed path. When the eighth experiment of Table II was repeated at 90 and 110°, the initial rates were 7.9×10^{-5} and 3.3×10^{-4} M/sec. By taking account of the temperature variation of the uncatalyzed path, the apparent activation energy for the catalyzed path was found to be 15.8 ± 0.5 kcal/mole.

The rate law found for the experiments in methanol was applied to the data obtained in acetone. Figure 1 is a plot of experiments done at constant porphyrin and Mg(II) concentrations and various pyridine concentrations. The plot is as expected for a two-term rate expression. If the H^+ concentration is assumed to be 1×10^{-8}, the rate constants found from intercept and slope are $k = 2 \times 10^{-8}$ M sec^{-1} and $k_2 = 4 \times 10^{-3}$ M^{-1} sec^{-1}. Experiments at different concentrations of Mg(II) and porphyrin gave slightly different values of the rate constants, possibly because the H^+ concentrations were not quite equivalent. The k values are comparable in magnitude to the corresponding values in methanol as solvent.

Reverse Reaction. In an attempt to determine whether pyridine also catalyzed the reverse reaction, *i.e.*, metal removal from porphyrin, the following experiments were performed.

The spectral changes as a function of pH (in the (a) absence and (b) presence of pyridine) of the Zn and Mg complexes of deuteroporphyrin diester were studied. The experiments were carried out in an essentially

Table II. Kinetics in Methanol at 100°

Concentrations, M				Initial rate, M/sec	k_1, M sec^{-1}	k_2, M^{-1} sec^{-1}
Porphyrin	Mg(II)	Pyridine	H$^+$			
1.7×10^{-5}	8.8×10^{-3}	0	3.2×10^{-8}	6.7×10^{-5}	1.3×10^{-7}	0
8.3×10^{-6}	8.8×10^{-3}	0	3.2×10^{-8}	3.3×10^{-5}	1.3×10^{-7}	0
1.7×10^{-5}	1.8×10^{-2}	0	3.2×10^{-8}	6.9×10^{-5}	1.3×10^{-7}	0
1.7×10^{-5}	4.4×10^{-3}	0	3.2×10^{-8}	6.8×10^{-5}	1.3×10^{-7}	0
1.7×10^{-5}	8.8×10^{-3}	0	4.0×10^{-7}	6.2×10^{-6}	1.5×10^{-7}	0
1.7×10^{-5}	8.8×10^{-3}	0	1.3×10^{-7}	1.8×10^{-5}	1.4×10^{-7}	0
1.7×10^{-5}	8.8×10^{-3}	1.3×10^{-2}	3.2×10^{-8}	1.2×10^{-4}	(1.3×10^{-7})	9×10^{-4}
1.7×10^{-5}	8.8×10^{-3}	2.5×10^{-2}	3.2×10^{-8}	1.7×10^{-4}	(1.3×10^{-7})	9×10^{-4}
1.7×10^{-5}	8.8×10^{-3}	5.0×10^{-2}	3.2×10^{-8}	2.7×10^{-4}	(1.3×10^{-7})	9×10^{-4}
8.3×10^{-6}	8.8×10^{-3}	2.5×10^{-2}	3.2×10^{-8}	8.9×10^{-5}	(1.3×10^{-7})	9×10^{-4}
4.2×10^{-6}	8.8×10^{-3}	2.5×10^{-2}	3.2×10^{-8}	4.6×10^{-5}	(1.3×10^{-7})	10×10^{-4}
8.3×10^{-6}	1.8×10^{-2}	2.5×10^{-2}	3.2×10^{-8}	1.6×10^{-4}	(1.3×10^{-7})	11×10^{-4}
8.3×10^{-6}	1.8×10^{-2}	2.5×10^{-2}	4.0×10^{-7}	1.5×10^{-5}	(1.3×10^{-7})	13×10^{-4}
8.3×10^{-6}	1.8×10^{-2}	2.5×10^{-2}	1.3×10^{-7}	4.0×10^{-5}	(1.3×10^{-7})	11×10^{-4}

aqueous environment by adding an aliquot of the metal complex in (a) acetone and (b) pyridine to a water solution of a nonionic detergent ("Tween-20", polyoxyethylene sorbitan monolaurate) to solubilize the porphyrin diesters in aqueous solution. HCl (0.2 M) was added dropwise to the metal-porphyrin solution; the pH was monitored with a pH meter, and a spectrum of the solution was taken promptly and at 1-unit intervals of pH. The results are summarized in Table III.

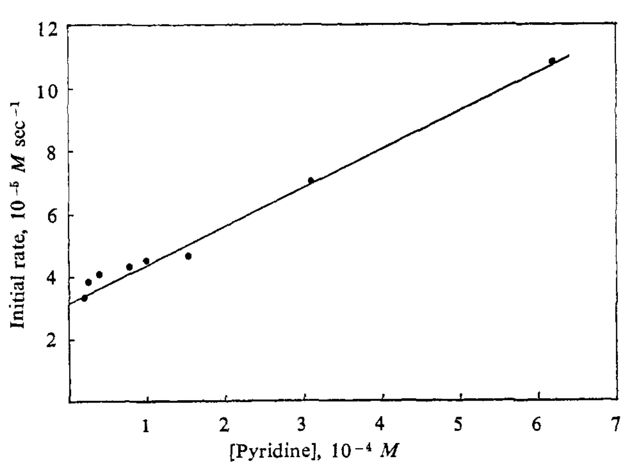

Figure 1. Plot of initial rate *vs.* pyridine concentration at constant porphyrin concentration (1.3×10^{-5} M) and constant magnesium concentration (2.2×10^{-2} M) in acetone.

Table III. Effect of pH, in the Presence and Absence of Pyridine, on the Removal of Metals from Porphyrin Diester

	Mg-deuteroporphyrin diester		Zn-deuteroporphyrin diester	
Species observed	Pyridine absent	Pyridine present	Pyridine absent	Pyridine present
Metalloporphyrin	pH >2	pH >5	pH >0	pH >4
Porphyrin	pH <2	pH <5	pH <0	pH <4

Discussion

Although pyridine is a catalyst for the incorporation of Mg(II) into porphyrins, the reaction is not specific for pyridine as noted above. Many nitrogenous bases having pK_a values between 4 and 7 serve as catalysts for the reaction. It is expected that such bases would bind Mg(II) to about the same extent as pyridine does, since pK_a values should provide an approximate guide to metal-ion affinity. A notable exception is 2,6-lutidene which has a pK_a only slightly less than the catalyst 2,4-lutidene, but owing to steric interference would not be expected to bind Mg(II) nearly as well. Some nitrogen bases having pK_a above 7 did not catalyze the reaction, possibly because they are so basic that they dissociate whatever water is present, thus causing the magnesium to precipitate out as $Mg(OH)_2$. (Analysis of the white precipitate formed in these latter reactions showed an intense band in the O–H region of the infrared spectrum.) This conclusion is further supported by the experiments with varying concentrations of hydroxide ion. Hydroxide ion in the concentration range of about 10^{-6} M does catalyze the reaction. However, when the hydroxide concentration is raised above 10^{-5} M, a white precipitate of $Mg(OH)_2$ appears and the rate of formation of Mg–porphyrin decreases.

For the mechanism of the metalloporphyrin formation reaction, previous workers have favored a displacement rather than a dissociation-type reaction.[6,7] Such mechanisms are supported by the identification of intermediate metal–porphyrin complexes.[3,8] In the present system, no evidence was found for appreciable concentrations of such an intermediate. The present rate law (at least for the uncatalyzed path) would indicate that the reaction can proceed by a prior dissociation mechanism.

The rate law found in the present work is consistent with either of the following mechanistic schemes for the uncatalyzed path

$$BH \rightleftharpoons B^- + H^+$$

$$B^- + PH_2 \longrightarrow PH^- + BH$$

$$PH^- + Mg^{2+} \xrightarrow{\text{rapid}} MgP + H^+$$

$$B^- = \text{base}, (CH_3O^-, OH^-, \text{etc.})$$

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(6) J. E. Falk, "Porphyrins and Metalloporphyrins," Elsevier Publishing Co., New York, N. Y., 1964.

(7) B. Dempsey, M. B. Lowe, and J. N. Phillips, "Haematin Enzymes," J. E. Falk, R. Lemberg, and R. K. Morton, Ed., Pergamon Press, New York, N. Y., 1961.

(8) E. B. Fleischer and J. H. Wang, *J. Am. Chem. Soc.*, **82**, 3498 (1960).

or

$$PH_2 \rightleftharpoons PH^- + H^+$$

$$PH^- \longrightarrow (PH^-)$$

$$(PH^-) + Mg^{2+} \xrightarrow{\text{rapid}} MgP + H^+$$

The inverse H^+ dependence of the rate observed in the pH range studied indicates the existence of an equilibrium involving the dissociation of some species having pK appreciably greater than 7, which could possibly be unavoidable, residual traces of H_2O. The alternate explanation is based on the equilibrium ionization of PH_2 followed by the rate-limiting, monomolecular step. The pK values of both PH_2 and PH^- have been given as being of the order of 16.[9] Consecutive dissociations at the pH studied of such weak acids would seem to be too slow to account for the observed rate. However, it is possible that, following the first ionization, a rate-limiting rearrangement of the anion PH^- could occur prior to a more rapid reaction with Mg^{2+}.

For the catalyzed path, the following sequence can be listed.

$$\text{pyr} + Mg^{2+} + PH_2 \rightleftharpoons \text{pyrMgPH}_2{}^{2+}$$

$$\text{pyrMgPH}_2{}^{2+} \rightleftharpoons \text{pyrMgPH}^+ + H^+$$

$$\text{pyrMgPH}^+ \longrightarrow \text{pyrMgP} + H^+$$

It should be noted that the initial proton removal from the ternary complex could instead be accomplished by B^- in the rate-limiting step. In either case the slow step involves proton removal from the pyridine–magnesium–porphyrin complex; the uncertainty lies in whether it is the first or the second proton which is rate limiting. Further alternatives seem much less likely. For example, the inverse H^+ dependence might be attributed to the pyridine–pyridinum equilibrium. However, pH measurements showed that the pyridine buffer region occurred at considerably more acid solution. Therefore, the pyridine is essentially completely as the free base in the reaction mixtures.

The proposed mechanism for the magnesium incorporation can be summarized by noting that in the absence of catalyst the rate-limiting step involves the proton removal from free porphyrin (possibly accompanied by internal rearrangement); in the presence of catalyst the rate-limiting step is again proton removal from porphyrin, but now from a pyridine–magnesium(II)–porphyrin complex. Apparently, coordinated magnesium aids in forcing off protons. The role of pyridine is to bring about formation of the reactive complex, since in the absence of pyridine the appropriate complex is either not formed, or is of the same reactivity toward base as the free porphyrin.

Direct evidence could not be obtained for a binary complex between pyridine and Mg(II). Stabilities of Mg(II) complexes with monodentate nitrogenous bases in water are apparently too low to measure, and even those with the bidentate ligands 2,2′-bipyridine and ethylenediamine are of relatively low stability.[10] On the other hand, there is evidence for a complex between pyridine and magnesium deuteroporphyrin. In solutions of very high pyridine concentration, all three spectral absorption bands shift to longer wavelength.[2,5] The kinetic importance of such a complex is indicated by the data of Table III which show that pyridine aids in the removal of metal ions from porphyrin.

The mechanism presented here is quite analogous to that presented for a similar reaction. Schiller and Bernauer[11] have studied the kinetics and mechanisms for the formation of metal complexes of phthalocyanine in aqueous solution. They find that the rate of incorporation of Cu(II) and Zn(II) is inversely proportional to the hydrogen ion concentration and is dependent upon the formation of a metal–base complex (here the base was hydroxide, pyridine, or picoline). In a rapid step, this metal–base complex forms an ion pair with the phthalocyanine which subsequently gives the metal–phthalocyanine.

The observed activation energies found must contain the temperature dependencies of all prior equilibria. However, for comparison, they are listed along with literature values[12] in Table IV. The uncatalyzed and metal-ion independent path which was found for Mg(II) should also be present for the other ions, where, however, it would be of undetectable importance. The catalyzed path, on the other hand, is energetically comparable to reaction paths for the other ions indicating that a change of the coordination sphere of Mg(II) is necessary in order to make it of comparable reactivity to that of other metal ions. That is, molecules such as pyridine are necessary in order to allow Mg(II) and and porphyrin to form the reactive intermediate which other metals can apparently form without pyridine.[8] In biological systems the Mg(II) might well be coordinated to a nitrogen base, the imidazole of histidine, for example. In fact, imidazole was found to be a catalyst comparable to pyridine.

Table IV. Activation Energies for Metalloporphyrin Formation

Ion	Energy, kcal/mole
Cu(II)[a]	10.2 ± 0.5
Cu(II)[b]	12.6 ± 0.5
Zn(II)[a]	15.9 ± 1.0
Co(II)[b]	17.4 ± 0.5
Mn(II)[b]	18.7 ± 3.0
Mg(II) catalyzed	15.8 ± 0.5
Mg(II) uncatalyzed	36.5 ± 0.5

[a] In 8.3% acetic acid, ref 12. [b] In glacial acetic acid, ref 12.

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