Involvement of Plasma Membrane Redox System in the Generation of Trans-Root Electrical Potential Difference in Excised Maize Root

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Abstract. Possible involvement of the plasma membrane bound redox system in the generation of the trans-root electrical potential difference (TRP) arising across 8 day old maize (*Zea mays* L hybrid ZPSC704) roots was studied Excised roots were exposed to artificial impermeable electron acceptors (potassium hexacyanoferrate III and potassium hexachloroiridate IV) in external solution, and TRP response oxygen consumption rate proton efflux and reduction of the electron acceptors were analyzed The effect of hexacyanoferrate III (HCF III) was tested at three concentrations (0 1, 0 5 and 1 0 mmol/l), and hexachloroiridate IV (HCI IV) in the concentration range 10^{-7} – 5 10^{-4} mol/l Both electron acceptors depolarized the trans-root potential an order of magnitude lower concentrations of hexachloroiridate producing a much more rapid depolarization of greater magnitude The roots had a higher capacity to reduce 0 1 mmol/l hexachloroiridate than 1 mmol/l hexacyanoferrate Also an increased level of acidification induced by HCI IV than HCF III could be observed The rate of oxygen consumption showed an increase of about 20% in both cases These results prove that electron transplasma membrane transport process(es) contribute to the total trans-root electrical potential difference across an excised maize root

Key words: Excised root — Hexachloroiridate (IV) — Hexacyanoferrate (III) — Plasma membrane electron transport — Trans-root potential — *Zea mays* L

Introduction

More than thirty years ago it was shown that an electron motive force (e m f)

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appears across plant roots, when measured by placing two macro-electrodes into the solutions in contact with the two ends of excised roots. First reports (Bowling and Spanswick 1964, Helmy et al. 1971) considered such a trans-root electrical potential difference (TRP) of roots as classical diffusion potentials appearing as a result of ionic concentration gradients and transport along the root. The use of metabolic inhibitors demonstrated that TRP is also linked to cellular metabolism and electrogenic processes in the root (Shone 1968, Davis and Higinbotham 1969, Radenovic et al. 1980). The electrogenic component of TRP is a resultant of at least two electrogenic processes, one being located at epidermal cell membranes and the other one at symplast/xylem interface (De Boer et al. 1983). Our study of the effect of metabolic inhibitors on TRP in maize roots indicated the existence of an electrogenic system contributing to the TRP to a greater extent than that would be obtained if the membrane ATPases were the sole contributor (Vuletic and Vučinic 1996). Since the existence of an electrogenic plasma membrane redox pump was postulated in 1980 (Ivankina and Novak 1980) and plasma membrane electron transport demonstrated by reduction of external artificial electron acceptors (Craig and Crane 1981), a number of studies have shown that membrane redox systems are a ubiquitous characteristic of all examined plant cells and tissues. Depolarization of membrane potential accompanying the reduction of external electron acceptors has been demonstrated in a number of cell types examined, including maize root cells (Doring et al. 1990, Doring and Bottger 1994). Such results are consistent with the presence of transplasma membrane electron transport system, suggesting the involvement of the plasmalemma redox system(s) in membrane energization.

The aim of this study was to prove that the electrogenic component of the trans-root electrical potential difference is also associated with the plasma membrane bound redox system of maize roots cells. We did this by exposing excised maize roots to artificial electron acceptors, potassium hexacyanoferrate III (HCF III) and potassium hexachloroiridate IV (HCI IV) in external solution and analyzing the TRP response, oxygen consumption rate, proton efflux and reduction of the electron acceptors.

Materials and Methods

Plant material

The experimental object used was the primary root of maize (*Zea mays* L. hybrid ZPSC704). After 3 days of germination at 25 °C, maize plants were grown for 5 days in aerated half strength Knopp solution in a controlled environment (12 h day 24/18 °C, 40 W m^{-2}, 75% RH). The roots were excised a day before the experiment and placed in lucite holders for TRP measurements. The cut end of the root was in contact with solution containing 100 mmol/l sucrose, 10 mmol/l KCl, 0.5 mmol/l

$CaCl_2$, pH 5.5, previously determined to be the optimal composition for sucrose replenishment in the lower part of the root (Vučinic and Vuletic 1995). The rest of the root was immersed in a non-buffered bathing solution containing 1 mmol/l KCl, 0.1 mmol/l $CaCl_2$.

Electrophysiological measurements

Trans-root potential measurements were performed simultaneously with oxygen consumption measurements using the arrangement as described in our earlier paper (Vučinic and Vuletic 1995). The measurements were performed on the excised and mounted roots after renewing the solution in the lucite holder in contact with the cut end of the root, and placing the roots into an experimental tube through which the bathing solution flowed at a rate of 1 ml min^{-1}. This bathing solution was substituted with a solution containing additional substances as shown in Results. The electron acceptors were added as soon as steady state TRP was obtained (usually in about 30 min). Potassium concentration was held constant during the measurements of TRP changes induced by potassium hexacyanoferrate III due to the strong potassium dependent effect on TRP diffusion potential (Helmy et al. 1971) and high concentrations of potassium added in the form of HCF III. The kinetic traces presented in the Results section are averaged results of 5–10 individual experiments, as explained in the paper by Vucinic and Vuletic (1995).

Oxygen consumption

Oxygen consumption by the root was measured during a period of temporarily stopped flow, prior to and following inhibitor addition and attainment of a new steady-state, by placing a Clark type O_2 electrode (Yellow Springs Instruments Co., Yellow Springs, U.S.A.) into the bathing solution.

Miscellaneous

All the measurements were performed at 25°C. The proton flux measurements and monitoring of reduction of artificial electron acceptors were performed by sampling the bathing solution into which three excised roots held by lucite holders were immersed. pH measurements were carried out in unbuffered bathing solution by means of a pH-meter. Plasmalemma electron transport reducing capacity (redox system activity) was determined spectrophotometrically (HP 8451 diode array spectrophotometer) by monitoring the concentration of oxidized forms of hexacyanoferrate and hexachloroiridate at 420 and 488 nm respectively. To exclude the possible effects of turbidity the measurements were compensated by values at 480 and 700 nm, respectively (Doring et al. 1990). Control experiments without plants were performed to compensate for possible side reactions with solution. The chemicals used were purity grade. Potassium hexachloroiridate was obtained from Aldrich Chem. Co.

Results and Discussion

Our recent results on the effects of metabolic inhibitors on the TRP (Vuletic and Vučinic 1996) demonstrated only a moderate effect of plasma membrane ATPase inhibitors on the initial phase of TRP depolarization as opposed to the rapid and pronounced effect of carbonyl cyanide *m* chlorophenylhydrazone *N*-ethyl maleimide and respiratory inhibitors. Assuming that the initial phase is mainly due to the effect on the cortical layer of the root cells and their plasma membranes, these results suggest the existence of membrane bound electrogenic system(s) contribution to the overall TRP and proton gradient to a greater extent than that accounted solely by the membrane ATPases.

The addition of either impermeable artificial electron acceptor HCI IV or HCF III induced depolarization of TRP (Figs. 1 and 3). HCI IV, possessing a much

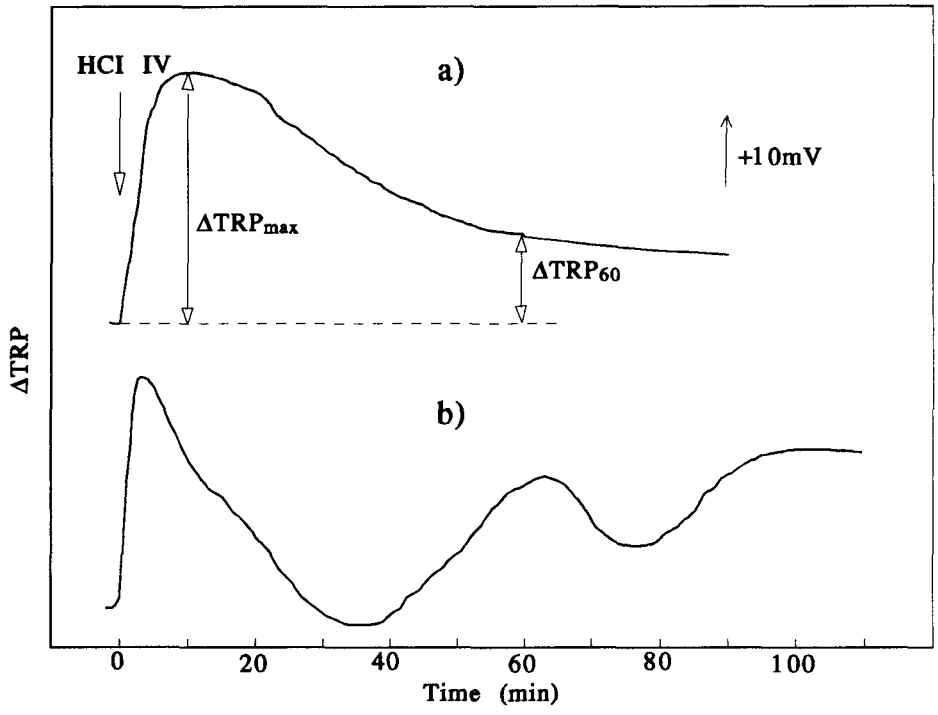

Figure 1. Examples of 0.1 mmol/l hexachloroiridate IV (HCI IV) induced changes in the trans-root electrical potential difference (TRP). Trace *a*) shows a typical time course of TRP depolarization with the two parameters numerically analyzed (ΔTRP_{max} maximal amplitude of initial depolarization, ΔTRP_{60} level of TRP depolarization attained 60 minutes after electron acceptor addition to the bathing solution). Trace *b*) gives an example of occasionally observed slow oscillations of TRP obtained following addition of electron acceptor.

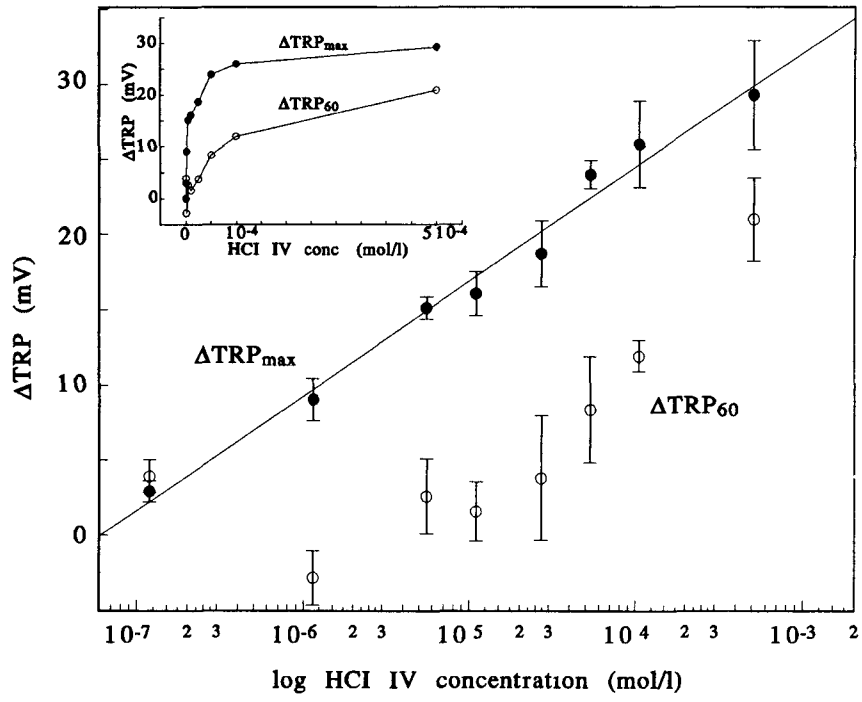

Figure 2. Concentration dependence of the effect of potassium hexachloroiridate IV on TRP (●) ΔTRP_{max} maximal amplitude of initial depolarization (○) ΔTRP_{60} level of TRP depolarization attained 60 minutes after electron acceptor addition

higher redox potential (+870 mV) induced depolarization which was much greater than that caused by HCF III (+360 mV) although the concentration of the former was one order of magnitude lower (0 1 mmol/l vs 1 mmol/l respectively) The rapid depolarization induced by HCI IV (4 4±0 5 mV min^{-1}) reaching a maximum within 5 10 min was followed by a partial or complete repolarization In the case of HCF III the initial depolarization was not as fast (1 2±0 2 mV min^{-1}) and subsequently did not repolarize to such an extent (at higher concentrations) The effect of HCF III on TRP was tested at three concentrations (0 1 0 5 and 1 0 mmol/l) Maximum depolarization of the initial phase induced by HCF III was 8 mV and it increased significantly only in the 0 1 0 5 mmol/l range (Fig 3) Concentration dependence of TRP changes induced by HCI IV was tested in the concentration range $10^{-7}-5\ 10^{-4}$ mol/l (Fig 2) The initial depolarization (ΔTRP_{max}) exhibited saturating kinetics with maximal depolarization of ~ 30 mV at saturating concentrations The concentration dependence of TRP depolarization measured after 60 min treatment (ΔTRP_{60}) did not exhibit a classical saturating kinetics and a

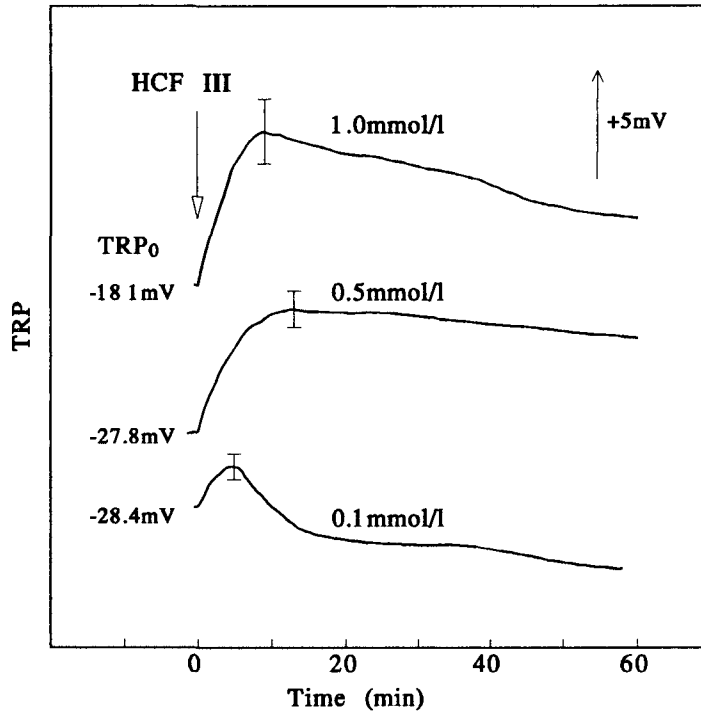

Figure 3. Kinetic traces of the effect of different concentrations of potassium hexacyanoferrate III on TRP. The concentration of K^+ in the bathing medium prior to and after acceptor addition was kept constant. The average values of steady state transroot potential (TRP_0) before HCF III addition are presented (5–10 experiments, the vertical bars in each of the traces showing ± S.E.)

statistically significant increase occurred above $5 \cdot 10^{-5}$ mol/l HCl IV (Fig. 2).

In some experiments TRP changes following electron acceptor addition exhibited oscillations with a period of ~ 40 minutes. A typical example is shown in Fig. 1*b*. Such oscillations of TRP observed in approximately 25% of all experiments, were more frequent at lower concentrations. The lowest concentration that induced TRP oscillation was 10^{-6} mol/l HCl IV. A possible explanation for the observed oscillations of TRP could be the rapid depletion of intracellular energy source contributing to the electrogenic plasmalemma redox system and its oscillatory renewal from the upper solution/transport pathways, characteristic for chained biochemical systems with a time lag.

The observed depolarization of TRP, induced by external electron acceptors that cannot permeate through plasma membranes, can be explained by electron transport through the plasma membranes of surface root cells. The magnitude of

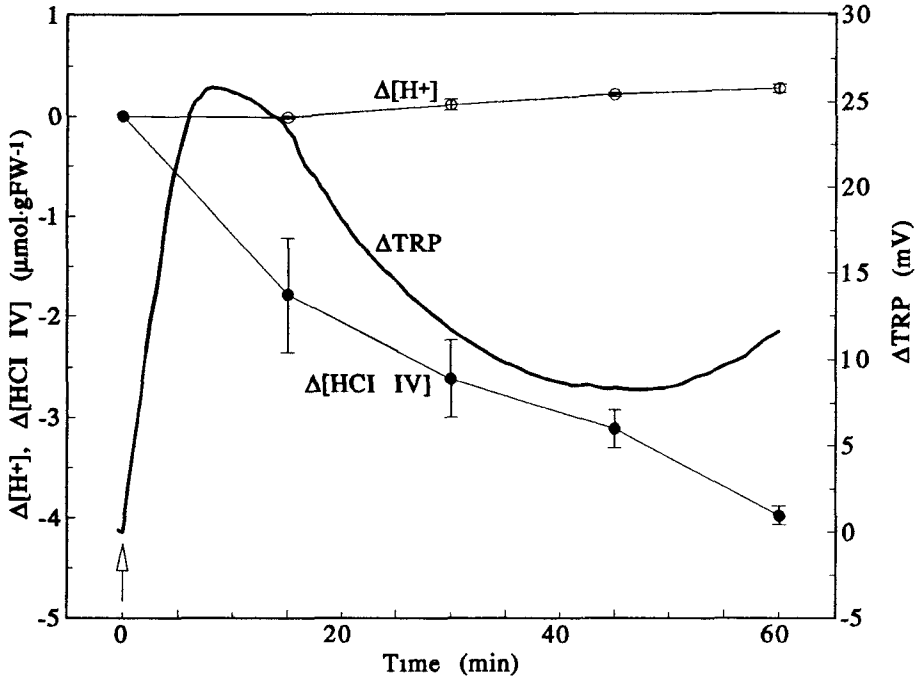

Figure 4. Results of parallel measurements of the effect of 0.1 mmol/l potassium hexachloroiridate IV on the change of trans-root potential (ΔTRP —) activity of the redox system(s) and proton extrusion. The activity of the redox system(s) was measured by determining the change in the concentration of oxidized form of hexachloroiridate due to its reduction by the root Δ[HCl IV] (●). Proton extrusion was determined as the change in proton concentration $\Delta[H^+]$ (○). Averaged curve (TRP) and averaged values with standard errors (indicated by vertical bars) are presented.

TRP changes induced by both acceptors used are close to those observed in intracellular microelectrode measurements of membrane potential changes performed on maize root cortex cells (Döring et al. 1990). These results provide direct evidence that redox process(es) occurring across plasma membranes of surface root cells participate in the generation of TRP.

The kinetics of TRP changes induced by HCl IV and associated reduction of HCl IV and proton extrusion by maize roots are shown in Fig. 4. It is obvious from the results presented that a higher rate of reduction of hexachloroiridate than of proton extrusion was obtained. Also, one could notice that TRP repolarization was followed by a gradual increase of proton extrusion and a slight decrease of electron acceptor reduction rate ~ 15 min following acceptor addition to the bathing medium. A comparison of the calculated rates of electron acceptor reduction and

Table 1 Reduction of electron acceptor (e^-) proton extrusion (ΔH^+) and stimulation of oxygen consumption rate after 60 min treatment with electron acceptors Oxygen consumption rate (v) is expressed as percent of the oxygen consumption rate before the addition of the acceptors (v_0)*

Treatment	e^- (mol g FW^{-1} h^{-1})	ΔH^+ (mol g FW^{-1} h^{-1})	O_2 $v/v_0 \times 100$	$e^-/\Delta H^+$ ratio
0 1 mmol/l HCF IV	(4 0 ± 0 1) 10^{-6}	(2 7 ± 0 4) 10^{-7}	120 ± 0 2	14 7
1 0 mmol/l HCF III	(2 7 ± 0 6) 10^{-6}	(3 6 ± 1 3) 10^{-8}	120 ± 3 0	76 7

* The average value of the rate of oxygen consumption by excised roots measured prior to acceptor addition (v_0) was 31 ± 6 μmol O_2 g fresh weight^{-1} h^{-1}

proton extrusion measured 60 min after addition of 1 mmol/l HCF III and 0 1 mmol/l HCF IV are presented in Table 1 The reduction of electron acceptors shown in our experiments was accompanied with only a slight stimulation of proton efflux and acidification of the bathing medium The observed rates of HCF III and HCF IV reduction by maize roots are of the same order of magnitude as those reported by other authors (Federico and Giartosio 1983 Qui et al 1985 Doring et al 1990) However the level of proton extrusion was low compared to the quantity of transferred electrons resulting in a high e^-/H ratio especially in the case of HCF III These results are different from the proton flux measurements performed on a number of plant objects including maize roots (Crane 1989) In the case of maize root segments it has been shown that HCF III-elicited a significant stimulation of potassium efflux (Kochian and Lucas 1985) This discrepancy of our result compared to those of other authors might be explained by charge balancing by other ion fluxes e g the involvement of potassium efflux Other possibilities include secondary proton uptake (possibly associated with transport of other compounds) difference in age use of excised roots variety of plants or bathing media etc Further experimentation is required to explain the observed difference

It is obvious that the roots had a higher capacity to reduce HCF IV than HCF III Also an increased level of acidification induced by HCF IV compared to HCF III could be observed Control experiments in which the rate of reduction of the acceptors was measured in solution after taking out the roots and shown to be of the same magnitude as that observed in the case of blank experiments performed in bathing solution without roots excluded the possibility that it were reducing agents excreted by roots that are responsible for such reduction The difference observed in the proton excretion and reduction of the two electron acceptors used can be explained by their different oxidation-reduction potentials, and their dissimilar ability to interact with the electron transport system at different sites A number

of different redox proton pumping domains have been postulated to function in series by Bottger and coworkers (Doring et al 1990, Bottger et al 1991), and the much stronger oxidizer HCI IV would be capable of pumping protons at a greater number of such proton loops than HCF III Such an explanation is also supported by our electrical measurements, in which HCI IV induced a greater change in TRP

Also included are the results of the measurement of oxygen consumption by the roots prior to and following the addition of electron acceptors The average rate of oxygen consumption, prior to electron acceptor addition, was 34 ± 6 μmol O_2 g fresh weight^{-1} h^{-1} and both HCF III and HCI IV treatment resulted in an increase of about 20% The increased oxygen consumption rate after treatment with electron acceptors obtained in our experiments, is contradictory to the model proposed by Bottger and Luthen (1986), where a decrease would be expected if oxygen was the natural electron acceptor competing for the reducing power with artificial acceptors The explanation for this contradiction could be sought in the involvement of some secondary process(es) increasing the oxygen consumption, since measurements of oxygen consumption in our experiments were performed at the end of the respective measurements (about 60 min after the addition of the electron acceptors)

A number of different proteins and enzymes have been shown to be able to transfer electrons, and in some cases protons, across plasma membranes, such as dehydrogenases, nitrate reductase oxidases, etc (see Bottger et al 1991) They are coupled to the intracellular metabolism mainly via reduced pyridine nucleotides, NADPH and/or NADH serving as intracellular electron donors It is also a well known fact that this plasma membrane bound redox system directly affects the cellular membrane potential difference The redox system was shown to affect the membrane potential of isolated plasma membrane vesicles (Hassidim et al 1987), individual cells (Thiel and Kust 1988) or multicellular plant tissues such as leaf (Beinstein et al 1989) or root (Doring et al 1990) etc The physiological role of the plasma membrane bound redox systems is still unclear Thus, it is thought to participate in the energization of the membranes, cell wall metabolism and regulation of plant development (Bottger et al 1991) However, what are the natural electron acceptors and where is the site of their electron acceptance (intracellular or extracellular) still remains unclear The most probable natural acceptors are nitrate semidehydroascorbate and oxygen

To conclude, our results have shown the involvement of trans-plasma membrane electron transport processes, induced by artificial non-permeable electron acceptors in the bathing medium washing the apoplast, in electrogenesis of the total electrical potential difference appearing at the two ends of a maize root Thus, a complex phenomenon such as TRP shown to be the result of a number of electrogenic processes in different root cells (Vuletic and Vučimić 1996), also has a component that is due to the participation of one or more of plasmalemma bound

redox systems The question that remains unresolved is what is the contribution of such redox plasma membrane reactions to net charge transfer *in vivo*, and what (if any) is the identity of the (possible) natural electron acceptors Our demonstration that TRP is directly linked to the plasmalemma bound electron transport and redox system of root cells means that the method of measurement of electrical potential difference across multicellular tissues such as root, hypocotyl or leaf makes it possible to study the physiological role of the redox system(s) and then coupling to as yet unidentified natural electron acceptors in a new and relatively simple manner

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