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CONF-9508202 QUINONE EXCHANGE AT THE A₁ SITE IN PHOTOSYSTEM I [PSI]

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A. Barkoff¹, N. Brunkan¹, S. W. Snyder¹, A. Ostafin¹, M. Werst¹,
J. Biggins² and M. C. Thurnauer¹, ¹Argonne National Laboratory, Argonne,
IL, 60439, USA; ²Brown University, Providence, RI, 02912, USA

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1. Introduction

Quinones play an essential role in light-induced electron transport in photosynthetic reaction centers (RC). Study of quinone binding within the protein matrix of the RC is a focal point of understanding the biological optimization of photosynthesis. In plant and cyanobacterial PSI, phylloquinone (K₁) is believed to be the secondary electron acceptor, A₁, similar to Q_a in the purple bacterial RC (1). Photoinduced electron transfer is initiated by reduction of the electron acceptor (A₀), a chlorophyll species, by the photoexcited primary donor *P₇₀₀. A₁ acts as a transient redox intermediate between A₀ and the iron-sulfur centers (FeS).

We have examined the characteristic PSI electron spin polarized (ESP) electron paramagnetic resonance (EPR) signal as a marker of the interacting radical pairs developed during electron transfer (2-5). The ESP EPR signal was assigned to the interacting radical pair, P₇₀₀⁺A₁⁻. The ESP EPR signal from PSI isolated from spinach was destroyed by K₁ extraction and restored by chemical reconstitution of K₁, either protonated, ^HK₁, or perdeuterated, ^DK₁ (2). When quinone-depleted PSI was reconstituted with ^DK₁, the ESP EPR signal exhibited linewidth narrowing and significant intensity changes indicating that K₁ was directly involved in the transient redox process (2). Further, the spectral shifts observed after reconstitution with ^DK₁ could be duplicated by incubation of PSI particles with ^DK₁ without prior extraction of the native K₁ (4). Attempts to exchange quinones lacking a hydrophobic phytol group such as 1,10-naphthoquinone (NQ) or 1,4-Cl₂NQ were unsuccessful (4).

Here we examine the conditions required for K₁ exchange in PSI isolated from the thermophilic cyanobacterium, *Synechococcus lividus*, and compare them with those required for PSI isolated from spinach. The advantage of the former system is that it is possible to study the exchange of ^HK₁ into deuterated PSI particles in addition to the exchange of ^DK₁ into protonated PSI particles, providing a complete data set that can be used for modeling. We have used the correlated radical pair polarization (CRPP) ESP model (6) to reproduce the observed lineshapes. Simulations allow us to determine whether the 'exchanged' quinone occupies the A₁ binding site in its native orientation, a perturbed orientation, or an alternate site generated as a consequence of the isolation procedure.

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2. Materials and Methods

PSI particles, (^HPSI, ^DPSI), were isolated from *S. lividus* (7). ^HK₁ was purchased from Aldrich and used without further purification. ^DK₁ was extracted from *S. lividus* (99.6 % deuterated). PSI preparations were incubated with K₁ dissolved in ethanol (100 μ M, 2% ethanol by volume). Control samples have PSI, 2% ethanol and no added quinones. ESP EPR signals were monitored by a light modulation, phase sensitive detection method as previously described (4). Light modulated signals are collected at 500 Hz, 0.01 mW microwave power and 1.25 G modulation amplitude.

3. Results and Discussion

The shape and intensity of the ESP EPR signal exhibited by a radical pair is dependent upon several factors, including: the time-dependence of electron transfer, spatial orientation, and isotopic composition of the involved species. Disruption of K₁ binding or other quinones in the A₁ site will perturb the ESP EPR signal (4,6,8).

The rates of quinone exchange depend upon incubation time and temperature. Figure 1 shows the time dependence of the exchange of ^HK₁ into ^DPSI at 50°C. The control sample exhibits an ESP EPR signal characteristic for the perdeuterated PSI (^DPSI). Quinone exchange in these samples produces an ESP EPR signal arising from ^DP₇₀₀⁺ ^HK₁⁻. The protonated component of this isotopically mixed case gives rise to a severely attenuated ESP EPR signal relative to the deuterated component. Thus, the exchange process is manifest by a diminution of the overall signal, particularly the low field part arising predominantly from quinone resonances (5,8,9). By this measure, 0.5 hr. incubation time at 50°C is sufficient for complete quinone exchange. At 30°C quinone exchange is partial even after 16 hr. incubation (data not shown).

The temperature dependence (0-50°C) for ^HK₁ incubated with ^DPSI is presented in Figure 2a. For each temperature an incubation period of 0.5 hr. is used. A transition from slow to fast exchange at a temperature between 40°C and 45°C is observed. Figure 2b shows exchange of ^DK₁ into ^HPSI. Incubation at 45°C for 0.5 hr. is required to produce the intense narrow signal characteristic of ^DK₁ in the site. We observed that the transition temperature for PSI particles derived from spinach is in the range 30-40°C. As a thermophilic bacterium, *S. lividus* would be expected to have a membrane composition optimized for survival at temperatures higher than for plant PSI. The primary adaptive mechanism of membranes to temperature is to shift the lipid composition to poised the gel-liquid crystal melting to slightly above ambient temperatures. We suggest that the difference in temperature-dependent transition observed for cyanobacterial and plant PSI is controlled by differences in the gel-liquid crystal melting temperature for the two cases. Further examination of this issue, as well as an investigation of the effect of detergent and solvent (10) on the exchange, are in progress.

The correlated radical pair polarization model (CRPP) for ESP can be used to approximate the observed lineshapes (6,11). Figure 3 shows the simulated spectra obtained for all isotopic variants. The electronic coupling parameters and the geometry for P₇₀₀⁺A₁⁻ used for the simulations have been previously described (6). The upper and lower inset show the contribution from 'P₇₀₀⁺' and 'K₁⁻', respectively. The changes observed in the experimental EPR spectrum of ^DPSI following incubation with ^HK₁, Figure 1, and ^HPSI incubated with ^DK₁, Figure 2b, are consistent with the differences

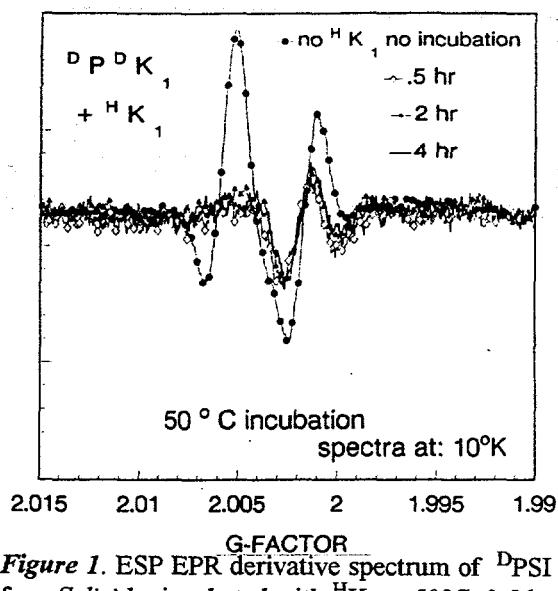


Figure 1. ESP EPR derivative spectrum of DPSI from *S. lividus* incubated with $^H K_1$ at 50°C: 0.5 hr (◊), 2 hrs (▲), 4 hrs (—). Control (●).

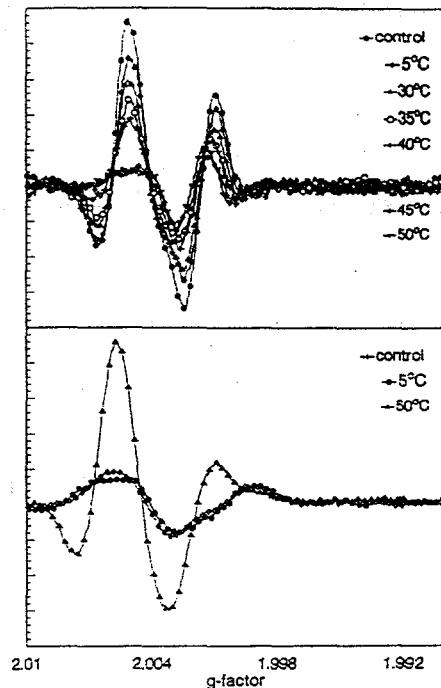


Figure 2. ESP EPR derivative spectrum of: (a) DPSI from *S. lividus* incubated with $^H K_1$ for 0.5 hr: 5°C (◊), 30°C (▲), 35°C (○), 40°C (+), 45°C (◊), 50°C (▽). Control (●). (b) HPSI incubated with $^D K_1$ for 0.5 hr: 5°C (●) and 50°C (▲). Control (◊).

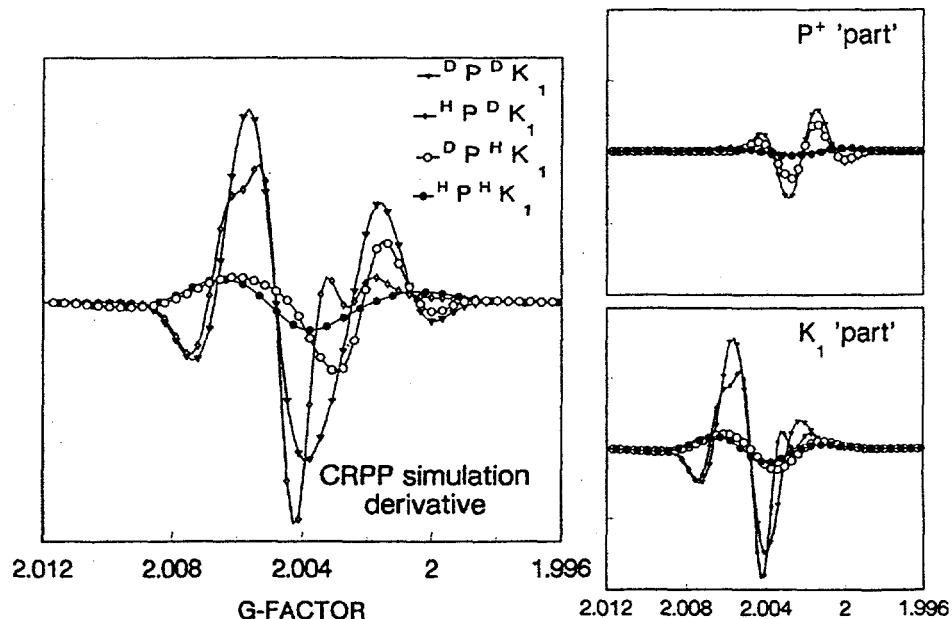


Figure 3. Simulated derivative EPR spectra for all isotopic variants ($^{DPD}K_1$, ▼/◊/ $^{DPH}K_1$, ○/ $^{HPH}K_1$, ●). Insets: contribution from 'P $^+$ ' and 'K $_1$ '.

observed in the simulated spectra with K_1 in the site. Variations in the dipolar angles, (not shown) indicate that the EPR spectrum is very sensitive to the orientation of the radicals in the site and suggest that the exchanged quinone is indeed in the site occupied by A_1 .

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References

1. Golbeck, J.H. and Bryant, D.A. (1991) in *Current Topics in Bioenergetics* (Lee, C.P., ed.), Vol. 16, pp. 83-177, Academic Press, New York.
2. Rustandi, R.R., Snyder, S.W., Feezel, L.L., Michalski, T.J., Norris, J.R. and Thurnauer, M.C. (1990) *Biochemistry* 29, 8030-8032.
3. Snyder, S.W., Rustandi, R.R., Biggins, J., Norris, J.R. and Thurnauer, M.C. (1991) *Proc. Natl. Acad. Sci., USA* 88, 9895-9896.
4. Rustandi, R.R., Snyder, S.W., Biggins, J., Norris, J.R. and Thurnauer, M.C. (1992) *Biochim. Biophys. Acta* 1101, 311-320.
5. Snyder, S. and Thurnauer, M.C. (1993) in *The Photosynthetic Reaction Center* (Deisenhofer, J. and Norris, J.R., eds.), Vol. II, Chapter 11, pp. 285-330, Academic Press, San Diego.
6. Kothe, G., Weber, S., Ohmes, E., Thurnauer, M.C. and Norris, J.R. (1994) *J. Phys. Chem.* 98, 2706-2712.
7. Biggins, J. and Mathis, P. (1988) *Biochemistry* 27, 1494-1500.
8. Van der Est, A., Sieckmann, I., Lubitz, W. And Stehlik, D. (1995) *J. Chem. Phys.* 194, 349-359.
9. Feezel, L.L., Gast, P., Smith, U. and Thurnauer, M.C. (1989) *Biochim. Biophys. Acta* 974, 149-155.
10. Warren, P.A., Golbeck, J.H. and Warden, J.T. (1993) *Biochemistry* 32, 849-857.
11. Morris, A.L., Snyder, S.W., Zhang, Z., Dutton, P.L., Robertson, D.E., Gunner, M.R. and Thurnauer, M.C. (1995) *J. Phys. Chem.* 99, 3854-3866.

times, information which can lead the inspectors to focus their attention on other sites. This difficulty illustrates the basic intellectual flaw in exclusive reliance on inspections and monitoring. Inspections and monitoring can never prove a negative, they can only determine where and when non-compliant activity takes place, not that it is not taking place.

Professional scientists, and most of the inspectors who have been utilized for the inspections in Iraq were drawn from their ranks, by nature and training are inclined to pursue solution of problems by the "scientific method", gathering data objectively and analyzing them to draw conclusions. However, in a possibly non-cooperative situation, under which inspections for non-compliance may well be conducted, effective inspections will have much more in common with forensic investigations than with technical data gathering. In order to gather the appropriate information, all leads, no matter how tenuous, and every indicator of prohibited activity need to be taken seriously and followed to their logical conclusions. This can be an expensive, time consuming and extremely frustrating experience. Further, techniques of data gathering and analysis appropriate to such a "forensic investigation" need to be taught to the inspectors. The question the inspectors must ultimately address is not whether the inspected party has an obvious, overt organization and program to pursue the proscribed activities, it is whether the inspected party is involved at all in any activities which have been prohibited by the international community. This requires the inspectors to utilize their basic scientific capabilities, and to add to that a meticulous attention to details.

Information obtained from within the inspected party via disenchanted workers or from outside the inspected party via defectors can be very useful. In the case of Iraq, leads developed as a result of such information channels proved to be the most helpful of all. While all such leads need to be carefully evaluated for authenticity, and to be followed up carefully, they can help illuminate entire areas of research previously effectively hidden or denied. Even when the lead does not illuminate a hidden or denied area, the very existence of fragmentary information can lead others within the system to come forward with new or more complete information. This was most dramatically demonstrated recently in Iraq when the defection of a senior member of the ruling cadre led to the release, by the Iraqi administration, of copious quantities of documents and information which had previously been declared as irretrievably lost and or destroyed. When it became apparent to the Iraqi leadership that the prior line of obfuscation was not going to remain tenable, a fall-back position of a more complete release of documentation was adopted. In many of the areas, it has been possible to examine this documentation for coherence and for completeness. Since the documentation is in Arabic, it must be translated in order to be confident that technical review is adequate, but some translation has been done, and some evaluation has been accomplished. This translation and evaluation is still underway but the initial finding is that this latest release of documentation is still far from complete. Thus, the conclusion of the finding is that this latest release of Iraqi documentation is also partial, and conforms to the pattern observed to date, namely that the Iraqis seem to release only enough documentation to address the current problem. The consequence of this is the inevitable conclusion they may have additional documentation they are unwilling to release, and this unreleased documentation may cover areas in which they are still trying to retain capability to resume proscribed activities.

In response to the failure of the Iraqis to make full disclosures and releases of documents, the inspectors had to assume that one of the goals of the current Iraqi administration was to retain as much capability as possible to resume their work on weapons of mass destruction as soon as the international situation allowed. If such capability were preserved, it could then be used to provide a foundation for the Iraqis to resume work on weapons of mass destruction if the political will of the international community should flag. Such an assumption allowed the inspectors to develop an appropriate program and to maintain the vigilance necessary to more effectively execute the mandate given to them by the international community.

LESSONS FROM THE IRAQIS

Among the lessons learned from the behavior of the inspected party in Iraq was that the presumption of accuracy, especially as applied to declarations and documentation, was misplaced. The Iraqis repeatedly gave false, incomplete and misleading declarations. In addition, when challenged, they often compounded this inadequate procedure with supplying "corrections" which included prior declarations "by reference", thus forcing the inspecting party to spend inordinately large amounts of time in deciphering and integrating the declarations. This technique, of distracting and delaying the inspecting party, was often used by Iraq.

Requests for documentation in support of declarations, or to reconstruct prior efforts on weapons of mass destruction, were often met by Iraqi declarations that such documentation was unavailable because it had been destroyed or lost. While there could have been some element of truth in these declarations, the inspectors often found enough documents and traces of documents that all such declarations of unavailability had to be questioned. The magnitude of the overall Iraqi effort to deceive was revealed first in 1991 in the discovery of about seven boxes of highly classified nuclear related documents by two of the early inspection teams. More recently, in 1995, the defection of high ranking persons in the Iraqi leadership led the Iraqi administration to release a large number of documents covering chemical weapons, biological weapons, long range ballistic missiles, and nuclear weapons. Many of these (more than a million pages of documents contained in 140 boxes plus an equivalent amount of microfilm) were related to the Iraqi nuclear weapons effort. This most unusual release happened after five years of the Iraqis insisting that all such documents had been destroyed in the early days after the Gulf War. What is also fully as astonishing as the release of the documents is that the documents themselves prove false many of the statements that the Iraqis had made during the five years of inspections. Most troubling though is that during this time the Iraqis apparently have continued to attempt to retain capabilities in all of the areas, and even to add to those capabilities, all this in the face of one of the most intrusive on-site inspection regimes the world has known.

Just as with the documents, many pieces of equipment and other material which had been procured as a part of the Iraqi nuclear weapons effort were declared to have been destroyed during the Gulf War, unilaterally destroyed by the Iraqis after the war, converted to civilian use after the war, or even lost due to the confusion resulting from the war and its aftermath. Subsequently, discoveries of equipment and supplies buried in the desert, observation that identification and nameplate information had been removed from equipment, and observation of deceptive modifications of equipment showed conclusively that the Iraqi declarations were often incomplete and designed to be deceptive. Most recently, new supplies of undeclared materials and components have been surrendered to the UNSCOM / IAEA inspectors as a part of the latest hand-over of nuclear related documents.

Frequently, weapons related buildings and facilities in Iraq were configured and designed to give the impression, at least externally, that only benign activities were taking place. Special care was often taken to minimize or alter emissions and external indicators for facilities where work on weapons of mass destruction was taking place. In the nuclear field, high efficiency particulate air filters (HEPA filters) are often used to eliminate or minimize emissions associated with work on radioactive materials, usually for environmental reasons. However, the Iraqis used such filters on many of their nuclear facilities, even out in the desert regions where environmental concerns might have been less. It may be that their primary motivation for such use was to reduce emissions to such a level that the facility and its activities could not be detected at a distance. This would assist materially in their efforts to hide such facilities.

The experience of the inspection parties in Iraq showed that the inspected party will often attempt to distract and subvert personnel of the inspection party. The Iraqis "hosted" them at luncheons, teas, and other social activities, even as a part of the inspections. They offered and conducted tours to historic sites. All these social activities and diversions served to distract the inspectors, and to reduce the time the inspectors could spend in actual inspections. The Iraqis would alternate this friendly, "good host" behavior with hostile behavior. They attempted to harass the inspectors by utilizing long travel times to sites to tire them out. Individual Iraqis (whose actions often seemed to be coordinated by the administration) exhibited antisocial behavior towards the inspectors when the inspectors were off duty, say eating at a local restaurant. Abusive and threatening telephone calls were often made to the inspector's rooms at all hours of the night.

The Iraqis also attempted to limit the inspector's rights of access, restricting them to only some sites within the country. They attempted to interpret the mandate of the inspectors in the most limited way possible. They sometimes used delaying tactics, such as driving to a site by a circuitous route, getting lost on the way, or professing confusion as to which site was intended for inspection. Such delays often resulted in "cleansing" of the site by the time the inspectors arrived, sometimes to the extent that all papers were gone from desks, book shelves and filing cabinets. When all else failed, the Iraqi hosts, under the pretense of attempting to protect the inspectors, would sometimes warn of danger at a particular site. This technique was used for sites which might pose exposure to physical danger as well as for sites which were particularly sensitive politically, such as Presidential palaces. Some sites which had been identified by defectors as "hide sites" were not inspected because of their particularly sensitive nature. Among these were private residences, cemeteries, and mosques or other holy places. Sometimes, when the inspectors would designate a site, the Iraqis would warn of the possibility of "spontaneous" demonstrations by the local populace which could expose some of the inspectors to physical danger. In one extreme case, shots were fired into the air to frighten off the inspectors.

Often, when asked to explain some unusual situation or discovery by the inspectors, the Iraqis would offer a simple explanation. If the inspectors requested further clarification, the Iraqis would often change or modify the explanation. Such a back and forth exchange could, and sometimes did go on a number of times, with the Iraqis offering new or modified "credible" explanations until they found one that the inspectors would accept. Sometimes, failing to get an answer, the inspectors would even abandon the particular line of questioning, and go on to other things. Patience and persistence are not only virtues, they are absolutely essential attributes for the inspectors.

Frequently, the Iraqi hosts, even while showing hospitality, would either ignore some questions or delay giving answers to them. They would deny their ability to produce answers or documentation which could support their assertions, claiming that the personnel involved in the activity were no longer available and that documentation had been destroyed. However, a number of incidents occurred over the course of the inspections where these professions of inability to produce the requested information and explanation were proven false. While there were many examples of these delays and evasions, probably the most dramatic was the revealing and surrender by the Iraqi administration in 1995 of a large number of documents related to the Iraqi research, development and production efforts on weapons of mass destruction. This release was a reversal of previously strongly stated positions. During five years of inspections the Iraqis insisted that all relevant documents had been destroyed in the Gulf War, or shortly thereafter at the direction of their administration! In all likelihood, the release of these documents would not have happened, even in 1995, except for the defection of a high ranking person in the Iraqi leadership. This defection seems to have led the Iraqis to the conclusion that they would be better off with the UNSCOM, the IAEA, and the United Nations Security Council if they revealed the documents and information rather

than have them come from a defector. As a result of the experience in the Iraqi inspections, it became clear that repeated failures on the part of the inspected party to answer questions, or requests for documentation must not be accepted as a sufficient reason for abandoning the requests. It may be that a tactical shift in the specific questions, or in the direction of the questioning is in order, but provision of the necessary data must continue to be pursued.

Probably the most disquieting outcome of the inspection process was that it appeared that the Iraqis learned a great deal from the focus and the execution of the inspections. Initially, both the Iraqis and the inspectors, inexperienced in the design and conduct of the inspections called for under UN document S/RES/687 (1991), were in a learning mode. As time went on, both sides became more sophisticated in their understanding of the inspection process and in their operations. It became increasingly apparent that the very questions the inspectors were asking, and the interest they showed in certain aspects of the inspections were telegraphing areas and points that the inspectors thought important in nuclear proliferation. Often it seemed that the Iraqi hosts would present information for the purpose of obtaining a reaction from the inspectors. This may have been an attempt to get a technical evaluation of their ideas and progress. In addition, the Iraqis became more adept at delaying the inspections, subtly diverting the inspectors from more important areas into less important areas, and introducing extraneous information and observations into the inspections.

LESSONS FROM THE INSPECTORS

Certain recurring themes became apparent as inspectors from many countries were gathered together to form the inspection teams and to carry out the inspections. Probably foremost in importance was the realization that the inspectors needed to be extremely knowledgeable in their own technical area. In many cases, Iraqi attempts to hide evidence and to obscure the relevant attributes of suspect facilities and equipment so confused the situation that only extremely capable and experienced inspectors could see through the attempts and arrive at knowledgeable conclusions. Many of the inspectors had had prior training in other inspection regimes (e.g. IAEA Safeguards inspections), but this training needed to be supplemented by additional exposure to other information and techniques.

The Security Council established the principle of an intrusive inspection regime in Iraq, and the roles of the UNSCOM and the IAEA very carefully. They established a system which drew on the strengths of past inspection experiences, but which went beyond these strengths in a number of important ways. One of the particular strengths that the UNSCOM and the IAEA had given to them by the Security Council was that the choice of which inspectors to send into the country lay completely with the UNSCOM and the IAEA. This procedure can be contrasted with the usual procedure, for example that followed by the IAEA in Safeguards inspections where the country to be inspected has the right to accept some inspectors and reject others. For the inspections in Iraq, the inspectors were chosen strictly on the basis of their technical capabilities, independent of their acceptability to the host country. The ability to choose the best people proved to be extremely important.

Careful preparation of the inspectors proved to be very helpful in fielding more adequate inspections. Frequently, the Iraqis would refuse to answer a question claiming that it had already been asked and answered. Sometimes they were right, but sometimes this ploy was used to evade the question, or to delay its answer. In preparation for the inspections, the inspectors attempted to learn all they could about prior inspections, the techniques that were used in gathering data, what were the results, and what were the remaining outstanding questions. Having this information at their fingertips helped make them and the inspection much more effective. Coupled with this learning and preparation process, careful analysis and

planning after the inspection was directed towards making future inspections more effective and better focused on the mission and tasking.

An important part of the training and preparation of the inspectors is what can be called *cultural acclimatization*. What has become abundantly clear over the course of a number of inspections is that the Iraqi culture seems to have a very different set of underlying presuppositions and a very different value system. The West, from where most of the inspectors came, is a basically technological society which places a high value on logic and reasoning. Further, it presumes a variety of underlying moral systems, each of which can affect behavior. Iraq seems to value the concept of "family" more highly than many in the West, and persons in Iraq often behave in ways which seem unusual to those whose roots are in the West. It seems that for many of the Iraqis the Baath party and the State have taken on the aura of "family", and actions individuals take can be better understood as actions taken to preserve and extend this family. It is not that either approach is inherently "good" or "evil", or that statements made by the participants can or cannot be trusted. Rather it is that the "drivers" in the different cultures are different. Within any given value system, behavior can be logical and can follow from the basic presuppositions. If, as was stated privately by one of the Iraqi counterparts¹⁰, maintenance of a nuclear weapons capability for Iraq was one of the highest priorities for Iraq, then obfuscation, delays, piecemeal doling out of information, and selective use of partial truths were not only understandable but were required by their value system. Once the behavior of the Iraqis was understood by the inspectors as coming from such a value system, it became possible to pursue the nuclear inspections and information gathering in a more effective way. The consequence of this realization was that all statements and exhibits offered by the Iraqis needed to be questioned and verified to a level that was different from the Western experience. Nevertheless, professional but skeptical behavior by the inspectors toward the Iraqi hosts often did lead to improved access and cooperation.

LESSONS ABOUT THE POLITICAL ASPECTS

On-site inspections in support of arms control agreements are carried out within a political environment. The terms and conditions of the inspections, and the support given to the inspectors are often conditioned more by political than by technical considerations. Whatever political environments led to the initiation of the inspections, strong continuing organizational support for the inspecting party continues to be essential. In the course of the nuclear inspections in Iraq, several times the international community came to the aid of the inspectors and their mission by threats of use of force, or actual use of force. In all cases, the behavior of the Iraqis seemed responsive to these external pressures. However, caution is necessary in use of forceful tactics. While threats of use of force and actual use of force seemed to encourage greater cooperation, the Iraqis had the sole responsibility for day to day safety of the inspectors. Even in the case of a defeated nation, with the resulting military disarray, the strength of the local military and police is such that it would be impossible to guarantee the physical safety of the inspectors by an external force.

Inspections and careful analysis of their results can provide information to the community which can serve as a kind of "trip wire", establishing a technical basis on which judgments of compliance or resumption of proscribed activities can be made by international political bodies. This process of inspections analysis and the absorption of the information by the international community occurred in the case of Iraq, especially with regard to the Iraqis attempting to hide equipment and material. As this information and the conclusions were brought to the attention of the United Nations Security Council, various mem-

bers of that body made demarches to the Iraqis, and attempted to lend support to the inspectors in other ways in order to encourage Iraq to behave more responsibly.

Finally, the experience of the international community in utilizing intrusive on-site inspections in Iraq demonstrated that technical on-site inspections can inhibit resumption of proscribed activities. However, manipulation of the inspectors and the inspection process by the Iraqi authorities has also shown that a determined evader can succeed in hiding documentation, activities, and capabilities. The intrusive inspection regime has materially affected the difficulty and the cost of conducting proscribed operations in Iraq. However it cannot prevent continued pursuit of the program, nor can it prevent continued theoretical work and planning. Such work can resume at any time, but especially if and when the zeal of the international community flags. The only way that long term behavior of Iraq can be permanently affected is through changes in its external and internal political conditions. In Iraq, one of the primary "drivers" seemed to be the presence of neighbors on virtually all sides who were perceived to be hostile. Another "driver" seemed to be a desire of the Iraqi leadership to take on a leadership role in the Arab world, and, if this leadership could be authenticated through possession of a nuclear weapons capability, then that is what they wanted to develop. To the best of our knowledge, none of these political-environmental imperatives have changed, and this seems to indicate the necessity for a continued, intrusive presence. The best that the international community can hope for, through the means of employing technical on-site inspections in Iraq, is that they can "buy a little time" so that appropriate political solutions can be developed and implemented, and that Iraq can be dissuaded in the future from pursuing work on nuclear weapons.

CONCLUSIONS

The on-site inspection regime authorized and instituted by the international community in Iraq in 1991 was extremely intrusive in its scope. As a unique situation, the experience gained in designing and executing this inspection regime can be invaluable. The extremely intrusive nature of the inspections, anytime and anywhere, is perhaps possible only in the case of a completely vanquished party, and may not be universally applicable.¹¹ Yet, some of the lessons learned in Iraq can perhaps be applied to other arms control regimes and situations, making them more effective. Lessons learned in the Iraqi inspections have already profoundly impacted the IAEA Safeguards control approach and techniques. The experiences in Iraq evidenced loopholes in the present system, and directly led to comprehensive studies on how to strengthen the system. The experiences in Iraq demonstrated the importance of a tighter control of exports and imports; they pointed to the vital importance of access to information gathered by national technical means; they have promoted the development of ad hoc surveillance instrumentation, supplying data in real time. The entire Safeguards system is being changed and made more effective; hopefully other arms control regimes will also adopt what is appropriate for them.

In today's world, no action is taken in a political void, and an intrusive on-site inspection regime is no exception. Every step of the process, from the initial establishment of the terms and conditions of the inspections to their design, execution and analysis is scrutinized for its political implications. However, whatever the political realities are for the establishment of the inspection regime, continued high level support for the process and for the inspectors is critical in order to effectively carry out the mandates of the international community. In the Iraqi case, direct intervention by the international community was required a number of times, at various levels of intensity. In some cases, a simple demarche was sufficient to convince the Iraqis to yield, while other cases required threats of use of force. A few times actual military force was needed to convince the Iraqi administration of the absolute nature of the requirements of its

complete compliance. Even with these demarches, threats, and actions when needed, Iraq continued to attempt to withhold information and to retain as much capability as possible to resume design, testing and manufacture of weapons of mass destruction. Given their local political situation, this was not only to be expected, it was almost required. What this means though is that the inspectors need to be fully aware of the political environment, and of the perceptions of the inspected party, and they need to adapt their behavior accordingly.

Probably the most important general lesson to be learned from the UNSCOM / IAEA experience in Iraq is that on-site inspections, while an essential part of many arms control scenarios, cannot prevent proliferation. However, they can introduce so much difficulty and cost into such a program that the potential proliferator is deterred, at least for a time. On-site inspections cannot prove the negative, absence of proliferation, they can only be used to observe and document suspect activity (or lack of suspect activity) at a given site at a given time. On-site inspections involve far more than objective gathering of data, and the analysis of it, they involve tenacious pursuit of every lead to its logical conclusion, no matter how tenuous it seems initially. They have far more in common with a forensic investigation than a simple data gathering exercise. In an on-site inspection regime, one of the best sources of leads to useful information is what is sometimes called "human intelligence". Information which comes from disillusioned workers within the inspected party, or from defectors outside the inspected party can lead to improved understanding, and new sites to inspect. Even in cases where the information from such sources is incomplete or misleading, it can be used to encourage others to come forward with additional information.

The behavior of the Iraqis under this intrusive on-site inspection regime initially appeared to be moderately cooperative. However, it soon became apparent that the preliminary assumption of the inspection teams, relative to the accuracy of the Iraqi declarations and documentation, was not a useful one. Many times during the course of the inspections, the Iraqis attempted to distract and delay the inspecting parties, and to mislead them with incomplete, fragmentary and false information. One of their favorite ploys was to declare that the requested information and documentation had been lost, misplaced or destroyed. The Iraqis used the same tactics when asked about specialized equipment and relevant material. These assertions were proven wrong many times, so many times that the preliminary presumption of the inspectors had to be changed from an attitude of "professional acceptance" to an attitude of "healthy skepticism". Frequently, buildings, facilities, and external emissions were configured and designed to convey the impression that only benign activities were taking place within. As the inspectors gained experience, and as they adopted this attitude of healthy skepticism, such efforts on the part of the Iraqis became less effective. Over the course of the inspections, repeated attempts were made by the Iraqis to limit access of the inspectors to a restricted set of sites in Iraq, and to limit the scope of the inspections. They would often profess confusion as to which site was to be inspected, delay access to the site, "clean up" the site prior to arrival of the inspectors, and in extreme cases would warn the inspection team leader of danger to the inspectors if they were to inspect a particular site. Thus, the inspectors need to be particularly aware of and sensitive to attempts to erode the rights of the inspectors. In addition, while professional collegiality may initially appear attractive, the inspectors need to conduct the inspections and the analysis on the basis of healthy skepticism.

Many of the UNSCOM / IAEA inspectors already had experience in designing and conducting on-site inspections. For example, for the IAEA inspectors these were experiences accumulated by them under the Safeguards inspection regime. Unfortunately, the constrained nature of Safeguards inspections made it difficult for some of the IAEA inspectors to adopt a forensic, adversarial posture. Some of the UNSCOM inspectors had prior experience in on-site inspections under some of the missile treaties or in trial inspections in preparation for the CW convention. Yet here too, the nature of the inspections was very different

from that in Iraq, and the transition was difficult. Initially both the Iraqi hosts and the UNSCOM / IAEA inspectors were inexperienced in the design and conduct of on-site inspections to be conducted from within so intrusive a structure as that defined by UN Document S/RES/687 (1991). Yet, while the prior experience of the Safeguards inspectors, the missile inspectors and the trial CW inspectors did not have much in common with the requirements of the Iraqi situation, one thing they did have in common was the necessity for the inspectors to have a deep and intimate familiarity with the technical aspects of the field. In many cases the Iraqi attempts to confuse the inspectors and hide relevant evidence was only surmounted through the insight of the inspectors. The other main attribute the inspectors need to have was to be very familiar with prior inspections, the results of prior inspections, and questions and answers from the Iraqi counterparts. Comprehensive data bases, easily accessible to the inspectors, are essential to a smooth operation and an effective transfer of information and data from one inspection team to another. Prior experience in participating in on-site inspections should not be assumed to have equipped an inspector to function adequately in very different situations, as in the Iraqi situation. It is essential to take advantage of their prior experience, but it is also necessary to supplement it with in-depth training and preparation. A successful inspection regime will require careful selection of inspectors, and then equipping them with all the resources they need to do the job.

Further, the inspectors need to be exposed to and carefully study differences and similarities between their own cultures and the culture of the inspected party. Only when the inspectors understand the underlying value system of the inspected party and their priorities can they adequately plan and execute an appropriate set of inspections and analysis. In the Iraqi situation, as time went on it became increasingly apparent that all statements and exhibits offered by the Iraqis needed to be questioned and verified to a degree much greater than many of the inspectors were used to. Compounding this difficulty was the necessity of coping with a clear attempt on the part of the Iraqis to retain as much of their weapons capability as possible. Five years of statements that all documents had been destroyed was nullified when, in 1995, due to high level defections of several Iraqis, the Iraqi administration decided to make available and release to the UNSCOM and the IAEA a large number of sensitive documents. It is clear that repeated failures on the part of the inspected party to answer questions or requests for documentation should never be accepted as sufficient reason to abandoning a given line of requests. It may be that a different line of questioning can lead to the desired result, and the inspectors need to be sensitive to the possible need for a change in tactics, but the goal should remain the same, to ascertain the truth. Patience and perseverance on the part of the inspectors is an absolute requisite.

What the above conclusions underscore is the absolute necessity to devise and employ a comprehensive inspector training program. The most effective inspectors were those who were experienced and who had taken part in comprehensive preparations for the inspections. Since there are many factors in common to all the inspection teams, it may be that a "curriculum" should be developed, and that all new inspectors should be required to complete it. Such a curriculum would certainly cover the technical aspects, but it could also cover forensic elements, political realities, and cultural aspects.

The experience in Iraq has demonstrated that a well thought through and executed on-site inspection system can inhibit resumption of proscribed activities. Having said this though, it still remains true that determined evaders can succeed in hiding evidence of malfeasance and resumption of prohibited activities. They can continue to plan and to conduct theoretical work, even while under the most intrusive inspection regime. The only way that the international community can encourage a proliferant country to move its long term behavior towards international norms is to openly address both the internal environment in the proliferator and its external environment.

Sometimes, as the case in Iraq, a charismatic leader can convince him/herself and his/her country that they have a manifest destiny to take on a major leadership role in the world. As a consequence, they may decide on a path of buildup of military might, including weapons of mass destruction. Similarly, if a potential proliferant country is convinced that its only route to national security lies in the development of weapons of mass destruction, then it should come as no surprise that this is the route it chooses. It may be that a future potential proliferator will learn from the Iraqi experience. It may be that they will delay their aggression until they have achieved their desired level of military might, and that they will elect another route to asserting their hegemony in their region. It may be that a simple demonstration of overwhelming power will be sufficient to convince their neighbors to acquiesce. One wonders what might have happened in the Mid-East if Iraq had delayed its aggression against Kuwait until Iraq had demonstrated its abilities in weapons of mass destruction.

While no on-site inspection regime, no matter how intrusive, can ever prevent proliferation, it can provide early warning. Discoveries by the inspectors, and careful analysis can serve as a "trip wire", establishing a basis on which the international community can arrive at determinations of compliance or non-compliance. Once having made these determinations, the international community can then decide on appropriate actions.

External and internal situations which can lead countries to embark on a self destructive course, as Iraq did in 1990, may well happen again in the future. Such situations must be monitored by the international community and the international community must take timely action to convince such countries that their best chance for national security lies in cooperation, not confrontation. Short term, on-site inspections can only inhibit resumption of proscribed behavior. For long term behavior modification, the external and internal political situations must be addressed.

REFERENCES

- ¹ The author is grateful to his many colleagues for sharing their valuable insights and suggestions. Special thanks are due to Dr. Kathleen Bailey who has been especially helpful. For this work, the author has drawn on many of the concepts Dr. Bailey set forth in her thoughtful book *The UN Inspections in Iraq, Lessons for On-Site Verification*, Westview Press, Boulder CO, 1995
- ² DORN, D.W., *Onsite Inspections in a Less Than Cooperative Environment: Nuclear Inspections in Iraq, a Case Study*, in *Missile Proliferation, Missile Defense, and Arms Control*, edited by Götz Neuneck/Otfried Ischebeck, Nomos Verlagsgesellschaft, Baden-Baden, Germany, 1993
- ³ The Director General of the IAEA has submitted seven reports on the implementation by the IAEA of the Plan for the Destruction, Removal or Rendering Harmless of Items Listed in Paragraph 12 of UN Document S/RES/687 (1991). The reports were circulated by the Secretary-General of the United Nations to the members of the Security Council in document UN Document S/23295, dated 17 December 1991; UN Document S/24110, dated 17 June 1992; UN Document S/24988, dated 17 December 1992; UN Document S/25983, dated 21 June 1993; UN Document S/26897, dated 20 December, 1993; UN Document S/1994/793, dated 5 July 1994; and UN Document S/1995/481, dated 13 June 1995. The Director General has also submitted eight reports on the progress made in implementation of the Ongoing Monitoring and Verification Plan. The reports, in order of the first through the eighth were circulated on 15 April 1992, as UN Document S/23813; on 28 October 1992, as UN Document S/24722; on 19 April 1993, as UN Document S/25621; on 3 November 1993, as UN Document S/26685; on 22 April 1994, as UN Document S/1994/490; on 10 October 1994, as UN Document S/1994/1151; on 11 April 1995, as UN Document S/1995/287; and on 6 October 1995, as UN Document S/1995/844.
- ⁴ The Sixth and Seventh IAEA Semi-Annual reports (UN Document S/1994/1151 of 15 October 1994 and UN Document S/1995/287 of 11 April 1995) contain comprehensive listings of detailed reports on most of the inspections.
- ⁵ United Nations, Department of Political and Security Council Affairs, UN Document S/RES/687 (1991), April 3, 1991.
- ⁶ International Atomic Energy Agency, Board of Governors, GOV/INF/609, May 2, 1991.
- ⁷ United Nations, Department of Political and Security Council Affairs, UN Document S/RES/707 (1991), August 15, 1991.
- ⁸ United Nations, Department of Political and Security Council Affairs, UN Document S/RES/715 (1991), October 11, 1991.
- ⁹ Dr. Edward Teller, private communication

¹⁰ Private communication.

¹¹ Note particularly the recent North Korean refusal of inspections by the IAEA Safeguards inspectors.

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