A possible role of rabbit heart cytosol tocopherol binding in the transfer of tocopherol into nuclei

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An α -tocopherol-binding macromolecule was isolated from the heart cytosol of rabbits fed for 1 month with an α -tocopherol-deficient diet. The amount of [3 H]-tocopherol bound to nuclear chromatin was increased when the α -tocopherol-deficient heart nuclei were incubated in the presence of [3 H]tocopherol–cytosol complex. In this condition, large amounts of [3 H]tocopherol were associated with a subnuclear fraction that contained non-histone acidic proteins.

Considerable work, published particularly by Nair and his co-workers (Hauswirth & Nair, 1972; Patnaik & Nair, 1975, 1977; Nair *et al.*, 1978), has shown that significant amounts of α -tocopherol are associated with the nuclei of rat liver. Experiments carried out *in vitro* and *in vivo* with [3 H]tocopherol have revealed the existence of a high-affinity nuclear tocopherol-binding receptor with biochemical characteristics of an acidic non-histone chromosomal protein (Patnaik & Nair, 1977; Nair *et al.*, 1978). Catignani (1975) and Catignani & Bieri (1977) have reported that rat liver cytoplasm contains a protein that binds [3 H]tocopherol with high affinity and specificity. Our recent research has demonstrated the presence of a cardiac cytoplasmic receptor for α -tocopherol (Guarnieri *et al.*, 1979). In the same study a considerable amount of α -tocopherol bound to heart muscle nuclei was also found. The purpose of the experiments described in the present paper was to estimate the role of α -tocopherol binding extracted from heart cytosol during the accumulation of the vitamin in the nuclei.

Materials and methods

Adult male New Zealand White rabbits (1.5 kg) were maintained on an α -tocopherol-deficient diet for 1 month (Young & Dinning, 1951). After the animals were killed, the hearts were immediately collected and then perfused for 60 min to eliminate blood contamination (Guarnieri *et al.*, 1978). Nuclei and chromatins from heart muscle were prepared as previously described (Caldarera *et al.*, 1975). Nuclei were washed in 10 vol. of cold 0.14 M-NaCl

and the chromatins were extracted from the nuclear pellets by homogenization in 10 vol. of 0.14 M-NaCl, 0.05 M-Tris/HCl, pH 7.5, with a Teflon/glass homogenizer, followed by stirring for 20 min and finally centrifugation at 15 000 g for 10 min. This extraction was repeated twice.

The isolation of the cytoplasmic receptor for [3 H]tocopherol from rabbit heart was obtained as described by Catignani (1975). Portions of the fat-free cytosol prepared by centrifugation at 105 000 g for 60 min were incubated at 4°C for 4 h in the presence of 100 nM-D-[5-methyl- 3 H]tocopherol (The Radiochemical Centre, Amersham, Bucks, U.K.). The cytosol was then immediately fractionated through a Sephadex G-100 column (2.5 cm $\times$ 50 cm) equilibrated with 50 mM-Tris/HCl, pH 7.5, 100 mM-KCl, at room temperature. Fractions (2.5 ml) were collected and analysed for radioactivity and protein (A_{280}). Saccharose (100 mM), pH 7.4, 45.0 nCi of [3 H]tocopherol–cytosol complex or free [3 H]tocopherol (45.0 nCi) was added to the nuclei suspended in 10 mM-sodium phosphate buffer, pH 7.4. Incubations were carried out for 120 min at 20°C, and values of accumulation of [3 H]tocopherol were measured in the chromatins isolated immediately after the incubations. The nuclear components were separated by dissolving the chromatins in 5 M-urea, 2 M-NaCl, 10 mM-Tris/HCl, pH 8.0; the acidic proteins were separated from the DNA–histone complex by dialysis as described by Patel (1974). For radioactivity estimation each sample was dissolved in Soluene (Packard Instrument Co., La Grange, IL, U.S.A.) at 50°C for 3 h and then added to a scintillation ‘cocktail’ (Insta Gel, Packard) before being measured in a Prias scintillator spectrometer (Packard).

Results and discussion

To separate the α -tocopherol binding we used an α -tocopherol-deficient heart, because considerably less binding was measured in the cytosol of rabbits fed with a stock diet as compared with the rabbits fed a tocopherol-free diet (Guarnieri *et al.*, 1979). The binding of [3 H]tocopherol *in vitro* to rat heart cytosol components is illustrated in Fig. 1. All of the radioactivity was eluted in the first protein peak, which comes immediately after the void volume. This fraction had a specific radioactivity of 58 760 d.p.m./mg of protein; radioactivity in other fractions was insignificant. The isolation of the cardiac cytosol tocopherol binding seems very similar to that found by Rajaram *et al.* (1973) in liver cytosol with the characteristics of α -tocopherol-binding protein. As shown in Fig. 2, in the system composed of isolated nuclei and free [3 H]tocopherol, the accumulation of the vitamin by the nuclei did not exceed 45 d.p.m./mg of protein. When [3 H]tocopherol was

Fig. 1. Association of [3 H]tocopherol *in vitro* with rabbit heart cytosol macromolecules

Gel filtration was carried out in a Sephadex G-100 column (2 cm $\times$ 50 cm) equilibrated with 50 mM-KCl, pH 7.5. Fractions were collected at room temperature and protein (—) and radioactivity (---) were determined.

added to the nuclei at the same concentration, but in the form of a complex with cardiac cytosolic carrier, the quantity of labelled tocopherol bound by the nuclear fraction strongly increased. The vitamin transported into nuclei could be active as an antioxidant. In fact, the nuclei are able to generate oxidative compounds such as superoxide anions or H_2O_2 (Bartoli *et al.*, 1977) that are agents known to decrease the DNA chain integrity (Nagai *et al.*, 1969; Onishi *et al.*, 1975) or to damage membrane structure (Halliwell, 1978). The further subfractionation of the chromatins after incubation with the labelled vitamin gave the results shown in Table 1. These results indicate that about 70–75% of the

Fig. 2. Uptake of [3 H]tocopherol *in vitro* by rabbit heart nuclei

The nuclei suspended in 2.0 ml of 10 mM-phosphate buffer, 100 mM-saccharose, pH 7.4, were incubated with 0.5 ml of free [3 H]tocopherol or [3 H]tocopherol-cytosol complex having the same radioactivity (45.0 nCi). The incubations were carried out at 20°C for the time periods indicated and the association of the vitamin with the chromatins was determined immediately after the incubations were stopped. The results are means $\pm$ S.E.M. for four separate experiments. symbols: $\blacksquare$, free [3 H]tocopherol; $\blacktriangle$, [3 H]tocopherol-cytosol complex.

Table 1. Intranuclear distribution of [3 H]tocopherol

The α -tocopherol-deficient nuclei were incubated with free [3 H]tocopherol (45.0 nCi) or [3 H]tocopherol-cytosol complex (45.0 nCi). The chromatins isolated after 1 h of incubation were fractionated as indicated in the Materials and methods section. The results are means $\pm$ S.E.M. for four separate experiments.

Nuclear fraction	Radioactivity (d.p.m./mg of protein)	
	Free [3 H]tocopherol	[3 H]Tocopherol-cytosol complex
Chromatin	35.3 $\pm$ 1.3	113.7 $\pm$ 9.8
DNA-histone complex	10.4 $\pm$ 0.9	26.6 $\pm$ 0.9
Non-histone acidic protein	24.6 $\pm$ 1.4	87.9 $\pm$ 4.0

original label was recovered in the solubilized acidic proteins, and confirm that the radioactive tocopherol is chromosome-associated. This result appears more consistent in nuclei incubated with [^3H]tocopherol bound to cytosolic complex. On the basis of the results reported in the present study, it is possible to propose that in order to accumulate α -tocopherol in the nuclei, it is first necessary to form a vitamin-cytosolic-barrier complex. This carrier is able to facilitate the translocation of the vitamin from the cytoplasm to the chromatin, where [^3H]tocopherol is preferentially bound to non-histone acidic proteins. Although no direct evidence is available regarding the role of the association of the vitamin with the nuclear acidic proteins of rabbit hearts, the fact that these proteins are involved in the regulation of gene activity (Allfrey, 1974) suggests that the vitamin may mediate cellular events at the nuclear level. Although this conclusion might be highly speculative for our study, the observations of Hauswirth & Nair (1972) and Patnaik (1978), who found in rat liver nuclei incubated with α -tocopherol a stimulatory effect on RNA synthesis, support the idea of an involvement of the vitamin in the control of gene activity.

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