Note

Effects of Carnosine and Anserine on the Destruction of Vitamin B_{12} with Vitamin C in the Presence of Copper

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Vitamin B_{12} is destroyed by the addition of substantial amounts of vitamin C in the presence of copper. Effects of carnosine and anserine, natural water-soluble antioxidants, on the destruction of vitamin B_{12}, were studied. Addition of carnosine (10 mm) effectively repressed the destruction of vitamin B_{12}, but anserine had only weak inhibitory effects.

Key words: anserine; antioxidants; carnosine; cobalamin; vitamin C

In multivitamin-mineral food supplements containing vitamin B_{12} (B_{12}), appreciable loss of biologically active B_{12} occurs since B_{12} is converted to B_{12} analogues by the addition of substantial amounts of vitamin C (VC) in the presence of copper. Some of the B_{12} analogues have been reported to block B_{12} metabolism in mammalian cells. The destruction of B_{12} is assumed to be concerned with radicals generated by VC in the presence of copper. Carnosine (β-alanyll-histidine) and anserine (β-alanyll-3-methyl-l-histidine) are present at high concentrations in animal muscles, some of which are used as foodstuffs (beef, pork, and chicken). Many biochemical studies have indicated that carnosine and anserine function as antioxidants in mammalian cells.

Here, we describe the effects of carnosine and anserine, natural water-soluble antioxidants found in foods, on the destruction of B_{12} with VC in the presence of copper and also discuss their use in preventing the destruction of B_{12} in multivitamin-mineral food supplements.

The reaction mixture (1.0 ml) contained 0.5 mM cyanocobalamin (CN-B_{12}) (Sigma, St. Louis, MO, U.S.A.), 10 mM VC (sodium salt) (Sigma, St. Louis, MO, U.S.A.), 1 mM CuCl_{2}, and the indicated amounts (0–10 nm) of carnosine or anserine (Sigma, St. Louis, MO, U.S.A.). The mixture was left for a week at 25°C in the dark and an aliquot (20 μl) of the mixture was quantitatively put on a pre-coated Silica gel 60 thin layer chromatography (TLC) plate (Merck, Darmstadt, Germany), which was developed with isopropanol–30% ammonium solution–water (7:1:2) as a solvent at room temperature in the dark. The TLC plate developed was dried at room temperature in the dark and analyzed spectrophotometrically with a Shimadzu Dual-wavelength TLC Scanner (CS-930) and Data Recorder (DR-2). The destruction of B_{12} was monitored by measurement of absorbance at 551 nm. The R_{f} of authentic CN-B_{12} was 0.55. The amount of CN-B_{12} in the VC-treated sample was expressed as a percentage of the control (without the VC-Cu^{2+} treatment).

Biological activity of the VC-treated CN-B_{12} was measured by the growth-supporting effect on B_{12}-requiring microorganisms, Euglena gracilis SM-ZK and Lactobacillus leichmannii ATCC 7830.

\[ \text{VC} \quad \text{Cu}^{2+} \quad \text{CN-B}_{12} \]

\[ \text{VC} - + + + \quad \text{Cu}^{2+} - + + + \quad \text{CN-B}_{12} \]

Fig. 1. Silica Gel 60 Thin Layer Chromatography of Vitamin B_{12} Treated with Vitamin C in the Presence or Absence of Copper. The reaction mixture contained 0.5 mM CN-B_{12}, 10 mM VC, and 1 mM CuCl_{2}, and was left for a week at 25°C in the dark. An aliquot of the mixture was quantitatively put on a silica gel 60 TLC plate, which was developed with isopropanol–30% ammonium solution–water (7:1:2) as a solvent at room temperature in the dark. Data represent a typical TLC pattern of the B_{12} treated by the VC-Cu^{2+} system from seven experiments. VC, vitamin C; Cu^{2+}, CuCl_{2}; -, absence; +, presence.

Abbreviations: B_{12}, vitamin B_{12} or cobalamin; CN-B_{12}, cyanocobalamin; VC, vitamin C or ascorbic acid.
Fig. 2. Effects of Concentrations of Carnosine and Anserine on the Destruction of Vitamin B12 by Vitamin C in the Presence of Copper.
Detailed procedures are described in the text. Relative content of B12 is expressed as percentage of the control (without the VC-Cu²⁺ treatment). •, carnosine; ●, anserine. All values represent mean ± SD (n = 5). Different letters denote significant differences (p < 0.05).

VC alone or metal ion (Cu²⁺) alone did not decompose CN-B₁₂. However, CN-B₁₂ was destroyed significantly by mixing both VC and Cu²⁺ (VC-Cu²⁺ system) (Fig. 1). Kondo et al.²¹ have reported that five B₁₂ analogues were isolated from a multivitamin-mineral pill by paper chromatography and that two of them slightly inhibit activities of hepatic B₁₂-dependent enzymes (methylmalonyl-CoA mutase and methionine synthase). In this study, lots of B₁₂ analogues (ladder-like red colored spots) were separated from the CN-B₁₂ treated with the VC-Cu²⁺ system by Silica gel 60 TLC so that we were not able to find main products among the B₁₂ analogues or identify any chemical properties of the B₁₂ analogues.

The extent of the destruction of CN-B₁₂ by the VC-Cu²⁺ system was estimated to be about 70% of the control (without the treatment) by TLC scanning (Fig. 2). The destruction of B₁₂ was reduced significantly by the addition of 10 mM carnosine or anserine; carnosine repressed the B₁₂ destruction more effectively (down to about 10% of the control) under these conditions than anserine (about 50%) did.

The extent of the destruction of CN-B₁₂ by treatment with the VC-Cu²⁺ system was also measured with E. gracilis, a B₁₂-requiring microorganism (Fig. 3A). About 70% of the biological activity of CN-B₁₂ was lost by treatment with the VC-Cu²⁺ system, but the activity of B₁₂ was retained up to about 98 and 75% of the control by the addition of 10 mM carnosine and anserine, respectively. Similar results were obtained with L. leichmannii (Fig. 3B). The results support the idea that carnosine and anserine repressed the destruction of B₁₂ by the VC-Cu²⁺ system as shown in Fig. 2, and also suggest that the B₁₂ analogues formed by treatment with the VC-Cu²⁺ system are biologically inactive for the B₁₂-requiring microorganisms.

Euglena cells cannot utilize B₁₂ analogues lacking the α-ligand (the cobalt-coordinated nucleotide) for cell growth,¹⁰ suggesting that some of the B₁₂ analogues formed by treatment with the VC-Cu²⁺ system are those lacking the α-ligand.

A variety of therapeutic effects (on ischemic heart damage, as an anti-inflammatory agent, treatment of cataracts, and so on) of carnosine have been found.¹⁹ Carnosine and anserine are known to act as strong pH-buffering agents, chelators of a variety of transition metal ions, and antioxidants protecting tissues from damage by free radicals.⁶ Decker et al.¹¹ have reported that carnosine (0.05–10 mM) is capable of inhibiting copper-catalyzed oxidation of VC because carnosine forms a complex with copper, which decreases its catalytic activity. These observations suggest that in protecting B₁₂ from destruction by the VC-Cu²⁺ system carnosine and anserine act as antioxidants or chelators for copper (or both).

The results presented here strongly suggest that addition of carnosine (10 mM) represses the destruction of B₁₂ in multivitamin-mineral food supplements.

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References


